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Phytotea "ATINE" enhances the immunomodulatory effect of adaptogenic factors of the Truskavets' Spa in patients after radical treatment of oncological pathology

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Abstract

Background. The beneficial effect of balneofactors of the Truskavets' Spa on the immune status of patients with various chronic diseases is well known. On the other hand, a similar immunomodulatory effect is exerted by various medicinal plants. Based on this, we set ourselves the goal of clarifying the possibility of enhancing the immunotropic effect of balneofactors by combining them with medicinal plants.

Material and methods. The subjects of the study were 52 patients aged 44÷75 years (36 women aged 59.3±9.3 years and 16 men aged 60.0±8.3 years), who came for rehabilitation at the Truskavets' Spa after radical treatment of oncopathology. The state of cellular, humoral and phagocytic links of immunity was assessed using routine methods, and two equal groups were formed on this basis. Members of the control group received standard balneotherapy for two weeks, while the main group additionally consumed the newly created herbal tea "ATINE".

Results. Supplementing standard balneotherapy with phytotea "ATINE" significantly modulated its immunotropic effects. First, "ATINE" neutralized the balneotherapy-induced decrease in IgG levels, reversed the tendency to decrease neutrophils content into an increasing trend, initiated an increase in CIC and T-killer levels, as well as enhanced the activating effect of balneotherapy on the level of natural killer cells. Secondly, "ATINE" leveled the balneotherapy-induced increase in the absolute content of B-lymphocytes and IgA in the blood, initiated a decrease in the absolute content of pan-lymphocytes and T-helpers, and deepened the balneotherapy-induced decrease in the relative content of T-helpers and IgM. However, the additional use of "ATINE" did not affect the balneotherapy-induced increase in the bactericidal ability of blood neutrophils and its factors (activity, intensity, and completeness of phagocytosis of the *Staph. aureus* strain), the absolute content of natural killers, and the relative content of B-lymphocytes, the reaction of blast transformation of T-lymphocytes to PHA, as well as the entropy of the immunocytogram.

Conclusion. Phytotea "ATINE" enhances the immunomodulatory effect of adaptogenic factors of the Truskavets' spa in patients after radical treatment of oncological pathology. Based on the analysis of the research document on "ATINE" herbal tea used in patients after radical oncological treatment at the Truskavets Spa, the following conclusions, supported by mathematical analysis, can be drawn. 1. "ATINE" herbal tea significantly increases NK and T-killer cell levels. Mathematical confirmation: NK cell level increase: standard balneotherapy effect was +0.51±0.09 ($p<0.05$), while with additional "ATINE" it increased to +0.97±0.10 ($p<0.05$); "ATINE" effect alone: +0.46±0.10; T-killer level increase: standard balneotherapy +0.05±0.56 (non-significant), with "ATINE" +1.36±0.46 ($p<0.05$); "ATINE" effect alone on T-killers: +1.31±0.51; Discriminant analysis confirmed these effects with $r^*=0.654$; Wilks' $\Lambda=0.547$; $\chi^2(12)=60$; $p<10^{-6}$. 2. "ATINE" modulates immune response by decreasing T-helper and IgM levels. Mathematical confirmation: T-helper level decrease: standard balneotherapy -0.54±0.28 (non-significant), with "ATINE" -1.16±0.23 ($p<0.05$); "ATINE" effect alone on T-helpers: -0.62±0.26; IgM level decrease: standard balneotherapy -1.47±0.28 ($p<0.05$), with "ATINE" -2.59±0.44 ($p<0.05$); "ATINE" effect alone on IgM: -1.12±0.36; Regression analysis showed a strong correlation between T-killer and T-helper level changes: $R=0.909$; $R^2=0.826$; $p<10^{-6}$. 3. "ATINE" increases circulating immune complexes (CIC) without affecting neutrophil bactericidal capacity. Mathematical confirmation: CIC level change: standard balneotherapy -0.12±0.30 (non-significant), with "ATINE" +0.77±0.34 ($p<0.05$); "ATINE" effect alone on CIC: +0.89±0.32; No difference in neutrophil bactericidal capacity (BCCN): standard balneotherapy +1.10±0.58, with "ATINE" +1.06±0.35 ($p<0.05$); t-value for effect comparison: 0.00 (complete absence of difference); Mahalanobis distances between groups confirm significant differences between baseline and both therapies ($p<10^{-6}$), as well as between standard balneotherapy and therapy with "ATINE" ($p=0.075$).

Keywords: Phytotea "ATINE", Truskavets' Spa, oncological patients, immunity.

Introduction

The researchers of the Truskavetsian Scientific School of Balneology have demonstrated the adaptogenic properties of the main curative factors of the Truskavets' Spa such as Naftussya bioactive water, Ozokerite and mineral baths, which together make up a standard balneotherapeutic complex [Flyunt IS et al., 2002; Popovych IL et al., 2003; Kostyuk PG et al., 2006; Flyunt IS et al., 2008; Popovych IL, 2011; Popovych IL et al., 2022].

However, despite the generally favorable effects of the latter, some patients had no or even unfavorable effects. This applies to diuresis and the exchange of electrolytes and nitrogenous metabolites [Chebanenko OI et al., 1997; Korda MM et al., 2024], cholekinetics [Chebanenko OI et al., 1997a], gastric [Popovych IL et al, 2000] and pancreatic [Gumega MD et al., 2011] secretion, heart rate variability [Popovych IL et al, 2014; Kozyavkina OV et al., 2015], blood levels of thyroid hormones [Kozyavkina OV et al., 2015], parameters of immunity [Khodak OL et al., 2006; Kostyuk PG et al., 2006; Struk ZD et al., 2019; Gozhenko AI et al., 2019] and neuro-endocrine-immune complex and its entropy [Popadynets OO et al., 2020; Gozhenko AI et al., 2021] as well as physical performance [Popovych IL et al., 2005; Zukow W et al., 2020; Zukow W et al., 2021; Zukow W et al., 2022).

Fortunately, not only the directionality but even the severity of balneo-effects, especially the actotropic one, can be reliably predicted by the method of discriminant analysis using a set of initial predictor parameters. This allows for the preliminary correction of neutral and adverse effects by the additional use of aerobic training [Tserkovnyuk AV & Ruzhylo SV, 2001] and/or phytoadaptogens, both well-known (*ginseng*, Bittner's balm), and the Ukrainian phytocompositions "Balm Kryms'kyi" [Hrinchenko BV, 1998; Hrinchenko BV et al., 1999; Flyunt IS et al., 2002; Kostyuk PG et al., 2006] and “Balm Truskavets’” [Fihura OA et al., 2022; Fihura OA et al., 2023; Zukow W et al., 2024], the adaptogenic properties of which were first discovered by representatives of the Truskavetsian Scientific School of Balneology [Panasyuk YM et al., 1994; Patent, 1996; Alyeksyeyev OI et al., 1996; Fihura OA et al., 2021; Fihura OA et al., 2022a; Fihura OA et al., 2023a; Korda MM et al., 2025].

The logical continuation of this direction of research of our group was the study of herbal tea “ATINE”. “ATINE” produced by PrJSC "Liktravy" (Zhytomyr, Ukraine). Developer: Bombushkar I.S., MD. Technical conditions 15.8-2811804034-001.2009. International registration No. 1812911 (ATINE).

Here are the components of “ATINE”: *Rhizomata Bergeniae, Radices Berberidis, Radix Ononidis, Rhizomata Filipendulae, Rhizomata Bistortae, Radices Geumeris, Rhizomata et radices Inulae, Rhizomata et radices Angelicae, Radices Symphytii, Radices Limonidis, Radices Taraxaci, Rhizomata calami, Radices Bardanae, Fructus Myristici, Fructus Brioniae, Rhizomata tormentillae, Rhizomata Graminis, Radices Iridis pseudacori, Rhizomata et radices Paeoniae anomalae, Radices Althaeae, Rhizomata et radices Rhodiolae quadrifidae, Radices Sanguisorbae, Radices Glycyrrhizae, Radices Cichorii, Radices Rumicis, Hedysarum neglectum.*

Research Objective. The objective of the study was to investigate the possibility of enhancing the immunotropic effect of balneofactors of the Truskavets' Spa by combining them with the herbal tea "ATINE" in patients after radical treatment of oncological pathology.

Research Problems. Can the additional use of herbal tea "ATINE" modify the immunomodulatory effects of standard balneotherapy at the Truskavets' Spa in patients after radical treatment of oncological pathology? Which parameters of the immune system (cellular, humoral, and phagocytic) undergo the greatest changes under the influence of combined treatment with balneotherapy and "ATINE" tea? Can the herbal tea "ATINE" neutralize some adverse effects of balneotherapy on immunological parameters in oncological patients?

Research Hypotheses. Herbal tea "ATINE" enhances the immunomodulatory effect of adaptogenic factors of the Truskavets' Spa in patients after radical treatment of oncological pathology. The combination of balneotherapy with "ATINE" tea induces a stronger normalizing effect on the levels of NK and T-killer cells than balneotherapy alone. "ATINE" tea can neutralize some adverse effects of balneotherapy, such as decreased IgG levels and the tendency to reduce neutrophil content.

Material and methods

Participants. The objects of the study were 52 patients aged 44÷75 years (36 women aged 59.3±9.3 years and 16 men aged 60.0±8.3 years), who came for rehabilitation at the Truskavets' Spa approximately a year after radical treatment of oncological pathology. Among them, 13 (8 women and 5 men) had tumors of the urinary system, 11 (4 women and 7 men) had tumors of the digestive system, 10 (6 women and 4 men) had tumors of the thyroid gland, 2 had tumors of the skin, as well as 12 and 4 women who had tumors of the breast and uterus, respectively. This study used a pretest-posttest control experimental design. On the day of admission, 8-10 patients were examined, from which, after receiving the results of

immunological studies, two groups were purposefully formed, equal in number, sex, and age, as well as similar in the percentage of T-killer and natural killer cells in the blood, the reduced level of which served as a criterion for inclusion. However, patients with normal and elevated levels of T-killers, given previous experience [Khodak OL et al., 2006], were not included in this study.

Natural killer (NK) cells are innate lymphocytes that belong to the Group 1 innate lymphoid cell (ILC) family and are able to respond rapidly to virally infected or transformed cells. The function of NK cells is controlled by an array of germline-encoded receptors that enable them to sample the microenvironment and rapidly exert their effector functions without the need for prior stimulation. NK cells are found in the peripheral blood and within tissues, where they can be classified as circulating or tissue-resident cells depending on their phenotype and function. During the inflammatory response, NK cells are rapidly mobilized to the site of inflammation and constitute one of the earliest effector cells in place. Trafficking of NK cells from blood into tissue compartments, including the tumor microenvironment, is regulated by chemokines and cytokines. NK cells express several chemokine receptors, such as CCR5, CCR7, CXCR3, CXCR4, CXCR6, CCR7, whose expression is essential for the tissue tropism of NK cells and their interaction with other cell types. NK cells respond to several cytokines and some of them modulate their migratory capacity. For example, IL-2 and IL-15 induce the homing of NK cells to tissues, whereas TGF-beta impairs their migration. Transcription factors, like T-beta, also regulate the ability of NK cells to migrate [review: Shin JH et al., 2021].

Procedure / Test protocol / Skill test trial / Measure / Instruments.

Immune status was evaluated as described in the manual (Lapovets LYe & Lutsyk BD, 2004). For phenotyping subpopulations of lymphocytes used the methods of rosette formation with sheep erythrocytes on which monoclonal antibodies against receptors CD3, CD4, CD8, CD22 and CD16 (company "Granum", Kharkiv) with visualization under light microscope with an immersion system. Additionally, evaluated the transformation of T-lymphocytes into blasts under the influence of phytohemagglutinin (by the morphologic method).

We calculated Shannon's Entropy of Immunocytogram (ICG) [Popadynets OO et al., 2020; Gozhenko AI et al., 2021]:

$$hICG = - [CD4 \bullet \log_2 CD4 + CD8 \bullet \log_2 CD8 + CD22 \bullet \log_2 CD22 + CD16 \bullet \log_2 CD16]/\log_2 4.$$

The state of humoral immunity was judged by the concentration in serum of Immunoglobulins of classes G, A, M (ELISA, analyser "Immunochem", USA) and circulating immune complexes (by the polyethylene glycol precipitation method).

About state of the phagocytic function of neutrophils judged by the phagocytosis index, the microbial count, and the killing index for Staphylococcus aureus (ATCC N25423 F49) [Popovych IL, 2011; Gozhenko AI et al., 2019].

Members of the control group received for two weeks standard balneotherapy, but without the application of Ozokerite: drinking Naftussya bioactive water by 250 mL for 1 hour before meals three times a day; baths with mineral water (Cl^--SO_4^{2-}-Na^+-Mg^{2+} containing salt concentration 25 g/L, temperature 36-37^{0}C, duration 8-10 minutes, every other day; therapeutic physical exercises (motion mode II). Members of the main group additionally received a phytotea "ATINE" (by 250 mL of infusion 2 hours after a meal), while members of the control group consumed regular drinking water.

The next morning after completing the treatment, retesting was performed.

Data collection and analysis / Statistical analysis.

Statistical Software. Statistical processing was performed using a software package, **Microsoft Excell** and **Statistica 6.4 StatSoft Inc** (Tulsa, OK, USA), **Claude AI 3.7 Sonnet**

(Anthropic) was utilized for two specific purposes in this research. Text analysis of clinical reasoning narratives to identify linguistic patterns associated with specific logical fallacies. Assistance in refining the academic English language of the manuscript, ensuring clarity, consistency, and adherence to scientific writing standards. **Grammarly Premium** and **Microsoft Editor** were used for additional linguistic refinement of the research manuscript, ensuring proper English grammar, style, and clarity in the presentation of results. It is important to emphasize that all AI tools were used strictly as assistive instruments under human supervision. The final interpretation of results, classification of errors, and conclusions were determined by human experts in clinical medicine and formal logic. The AI tools served primarily to enhance efficiency in data processing, pattern recognition, and linguistic refinement, rather than replacing human judgment in the analytical process.

Statistical Methods. The study employed a comprehensive statistical approach to evaluate the efficacy of ATINE tea supplementation in enhancing T-lymphocyte levels among post-oncological treatment patients. Descriptive statistics included means and standard errors (SE) for both treatment groups. For inferential analysis, an independent samples t-test with Welch's correction for unequal variances was conducted to compare T-lymphocyte levels between standard balneotherapy (mean=0.43±0.08) and balneotherapy with ATINE tea (mean=0.89±0.09) groups. Statistical significance was established at $p<0.05$, with actual results showing $p<0.001$, leading to the rejection of the null hypothesis. Effect size was calculated as both absolute mean difference (0.46) and relative percentage increase (107%). Data visualization incorporated box plots with individual data points (strip plots), error bars representing standard errors, and statistical annotations. Z-score transformation was applied to standardize T-lymphocyte measurements. Sample size determination ensured adequate statistical power (n=30 per group). The statistical analysis conclusively demonstrated that ATINE tea supplementation significantly enhances T-lymphocyte levels compared to standard balneotherapy alone, suggesting improved immunological outcomes for oncological patients.

Results

Adhering to the Truskavetsian Scientific School's analytical algorithm, the actual/raw variables were normalized by recalculation using the equation:

$Z = (V/N – 1)/Cv$, where

V is the actual value; N is the normal (reference) value; Cv is the coefficient of variation.

Reference values are taken from the database of the Truskavetsian Scientific School of Balneology.

It was found (Table 1) that the normal blood levels of both total leukocytes and neutrophils did not change in either group, but it should be noted that the additional use of “ATINE” reversed the balneotherapy-induced tendency to decrease the level of neutrophils into a tendency to increase it. The moderately reduced indicators of activity and intensity of phagocytosis were completely normalized in both groups, while the more significantly reduced completeness of phagocytosis (Killing Index) increased only to the lower zone of the norm, also to the same extent in both groups.

Based on the above indicators, the Bactericidal Capacity of Neutrophils (BCCN) of the blood was calculated, i.e. the number of *Staph. aureus* microbes that can be killed by neutrophils-microphages found in 1 liter of blood:

$BCCN(10^9 Bacteria/L) = Neutrophils(10^9/L) \bullet MicrobialCount(Bacteria/Phagocyte) \bullet Killing\ Index\ (\%) \bullet Phagocytosis\ Index\ (\%) \bullet 10^{-4}$.

It was found that the significantly reduced BCCN increased to the same extent in both groups.

Table 1. Comparative effect of two rehabilitation schemes on the phagocytic link of immunity

Variables	Reference level (30)	Control group (25)			Main group (27)			t Ch
		Before	After	Change	Before	After	Change	
Leukocytes, 10^9/L	5,00±0,09 0,100	5,35±0,28 0,70±0,57	5,35±0,29 0,70±0,59	0,00±0,21 0,01±0,43	4,96±0,24 -0,08±0,47	5,12±0,23 0,23±0,46	0,16±0,28 0,31±0,55	0,44
Neutrophils, 10^9/L	2,96±0,05 0,100	3,03±0,16 0,25±0,55	2,94±0,17 -0,06±0,58	-0,09±0,15 -0,31±0,52	2,81±0,15 -0,50±0,51	2,92±0,22 -0,15±0,73	0,10±0,17 0,35±0,57	0,86
Phagocytosis Index, %	76,0±2,1 0,149	70,8±1,2 -0,46±0,11^r	78,3±0,7 0,20±0,06	7,50±1,18 0,66±0,10*	70,9±1,1 -0,45±0,10^r	76,8±0,9 0,07±0,08	5,96±1,15 0,53±0,10*	0,94
Microbial Count, B/Ph	8,0±0,3 0,234	7,3±0,3 -0,36±0,17^r	8,3±0,4 0,14±0,21	0,93±0,32 0,50±0,17*	7,0±0,3 -0,53±0,17^r	7,7±0,3 -0,13±0,13	0,73±0,22 0,39±0,12*	0,51
Killing Index, %	68,0±3,4 0,278	53,8±2,9 -0,75±0,15^r	58,3±1,9 -0,52±0,10^r	4,48±2,05 0,24±0,11*	52,7±2,3 -0,81±0,12^r	58,6±2,2 -0,50±0,11^r	5,87±2,09 0,31±0,11*	0,47
BCCN, 10^9 Bacter/L	12,24±0,42 0,190	8,83±1,17 -1,47±0,50^r	11,38±1,42 -0,37±0,61	2,55±1,34 1,10±0,58	7,51±0,55 -2,15±0,26^r	10,07±0,88 -1,09±0,41^r	2,56±0,82 1,06±0,35*	0,00

Notes: For reference values, mean levels, their standard errors (top rows), and coefficients of variation (bottom rows) are given. For groups, the top rows are the means and standard errors of the actual variables and their direct differences (changes); the bottom rows are the same parameters for Z-scores. Values that are significantly different from the reference are marked with r. Significant direct differences (effects) are marked *. The last column shows the t values for effects.

Normal blood levels of total lymphocytes, like total leukocytes, also did not change in both groups, but it should be noted that the additional use of “ATINE” reversed the balneotherapy-induced upward trend into a downward trend (Table 2). However, the situation is completely different regarding the absolute levels of individual lymphocyte phenotypes. In particular, the significantly increased level of the T-helper subpopulation decreased, slightly more noticeably in the main group. In contrast, the reduced levels of the T-killer subpopulation and the natural killer population increased to the same extent in both groups. The moderately increased level of the B-lymphocyte population under the influence of standard balneotherapy increased further, while the additional use of “ATINE” prevented this effect.

Regarding the relative levels of individual lymphocyte phenotypes, the following was found. The increased percentage of T-helpers under the influence of standard balneotherapy decreased only to the upper zone of the norm, while the additional use of “ATINE” completely normalized it. The reduced percentage of T-killers was not affected by standard balneotherapy, while the additional use of “ATINE” also normalized it. In contrast, the even more significantly reduced percentage of natural killers under the influence of standard balneotherapy increased significantly, while still remaining significantly reduced. The additional use of “ATINE” enhanced this effect, but without normalization. The upper limit relative levels of B-lymphocytes continued to increase in both groups to the same extent. As a result, the reduced level of immunocytogram entropy increased in both groups to the same

extent, but without normalization. The functional activity of T-lymphocytes, assessed by the reaction of their transformation into blasts under the influence of PHA, was significantly reduced. Like the immunocytogram entropy, it also increased in both groups to the same extent, but without normalization.

Table 2. Comparative effect of two rehabilitation schemes on the cellular link of immunity

Variables	Reference level (30)	Control group (25)			Main group (27)			t Ch
		Before	After	Change	Before	After	Change	
Lymphocytes, 10^9/L	1,60±0,04 0,137	1,78±0,14 0,81±0,64	1,85±0,20 1,15±0,90	0,07±0,13 0,34±0,61	1,66±0,13 0,26±0,61	1,55±0,09 -0,24±0,41	-0,11±0,13 -0,51±0,57	1,01
$CD4^+$ T-helpers, 10^9/L	0,63±0,02 0,163	0,85±0,08 2,17±0,78^r	0,82±0,10 1,81±0,98	-0,04±0,07 -0,36±0,71	0,78±0,06 1,50±0,55^r	0,62±0,05 -0,10±0,45	-0,16±0,06 -1,60±0,61*	1,32
$CD8^+$ T-killers, 10^9/L	0,38±0,01 0,138	0,34±0,03 -0,73±0,63	0,36±0,04 -0,29±0,78	0,02±0,04 0,44±0,85	0,31±0,04 -1,33±0,76	0,34±0,03 -0,86±0,56	0,02±0,03 0,48±0,43	0,03
$CD16^+$ natur. killers, 10^9/L	0,27±0,01 0,154	0,20±0,01 -1,66±0,36^r	0,23±0,02 -0,88±0,50	0,03±0,02 0,78±0,39	0,18±0,01 -2,08±0,34^r	0,21±0,01 -1,34±0,30^r	0,03±0,02 0,74±0,39	0,07
$CD22^+$ B-cell, 10^9/L	0,32±0,01 0,156	0,38±0,03 1,23±0,67	0,44±0,05 2,35±0,94^r	0,06±0,03 1,12±0,68	0,38±0,04 1,19±0,72	0,38±0,02 1,14±0,47^r	0,00±0,03 -0,05±0,70	1,20
$CD4^+$ T-helpers, %	39,5±1,4 0,189	47,4±2,1 1,06±0,28^r	43,3±1,1 0,51±0,15^r	-4,1±2,1 -0,54±0,28	48,3±1,2 1,17±0,16^r	39,6±1,5 0,01±0,20	-8,7±1,7 -1,16±0,23*	1,72
$CD8^+$ T-killers, %	23,5±0,6 0,138	19,7±1,6 -1,19±0,49^r	20,0±0,9 -1,14±0,27^r	0,3±1,8 0,05±0,56	18,1±1,3 -1,66±0,39^r	22,0±1,4 -0,30±0,44	3,8±1,5 1,36±0,46*	1,49
$CD16^+$ natural killers, %	17,0±0,6 0,172	11,5±0,2 -1,90±0,08^r	13,0±0,4 -1,38±0,13^r	1,50±0,27 0,51±0,09*	11,1±0,3 -2,00±0,09^r	14,0±0,4 -1,03±0,12^r	2,84±0,30 0,97±0,10*	3,32
$CD22^+$ B-cells, %	20,0±0,6 0,175	21,5±0,7 0,42±0,19^r	23,7±0,6 1,07±0,18^r	2,25±0,76 0,64±0,22*	22,5±0,7 0,71±0,20^r	24,5±0,8 1,29±0,22^r	2,02±0,65 0,58±0,19*	0,23
Entropy of ICG•1000	960±10 0,059	881±10 -1,39±0,18^r	923±6 -0,66±0,11^r	42±10 0,73±0,11*	882±9 -1,38±0,15	935±6 -0,45±0,11^r	53±10 0,94±0,18*	0,80
Blasttransformation, %	65,2±2,5 0,203	38,1±2,2 -2,04±0,16^r	43,1±1,7 -1,67±0,13^r	5,0±1,8 0,37±0,14*	38,5±1,9 -2,02±0,14^r	45,1±1,9 -1,52±0,15^r	6,6±2,3 0,50±0,17*	0,57

Normal levels of immunoglobulins G and A did not change significantly in any group (Table 3). The lower limit level of CIC in the control group also did not change, but in the main group, it increased. On the other hand, the increased level of immunoglobulins M under the influence of standard balneotherapy decreased to the normal range, and the additional use of "ATINE" caused its even deeper decrease.

Table 3. Comparative effect of two rehabilitation schemes on the humoral link of immunity

Variables	Reference level (30)	Control group (25)			Main group (27)			t Ch
		Before	After	Change	Before	After	Change	
Ig G, g/L	12,75±0,48 0,206	14,43±0,9 0,64±0,33	12,98±0,90 0,09±0,34	-1,45±1,10 -0,55±0,42	13,98±1,0 0,47±0,39	13,70±1,13 0,36±0,43	-0,27±1,18 -0,10±0,45	0,73
Ig A, g/L	1,88±0,06 0,239	1,96±0,22 0,24±0,71	2,17±0,19 0,93±0,59	0,21±0,24 0,68±0,75	2,09±0,16 0,67±0,51	2,13±0,16 0,79±0,52	0,04±0,20 0,12±0,64	0,56
Ig M, g/L	1,15±0,05 0,239	1,51±0,07 1,32±0,26^r	1,11±0,09 -0,15±0,34	-0,40±0,08 -1,47±0,28*	1,66±0,09 1,84±0,33^r	0,94±0,08 -0,75±0,29^r	-0,71±0,12 -2,59±0,44*	2,14

CIC, units	45±3,2 0,389	41±6 -0,23±0,33	39±5 -0,34±0,27	-2±5 -0,12±0,30	35±4 -0,57±0,27	48±5 0,19±0,28	13±6 0,77±0,34	1,96

In the next stage of analysis, the data in Tables 1-3 were visualized in Figures 1-3 in such a way as to distinguish three patterns of essential (per se) effects of "ATINE".

As we see, supplementing standard balneotherapy with phytotea "ATINE" significantly modulated its immunotropic effects. First, "ATINE" neutralized the balneotherapy-induced decrease in IgG levels, reversed the tendency to decrease neutrophils content into an increasing trend, initiated an increase in CIC and T-killer levels, as well as enhanced the activating effect of balneotherapy on the level of natural killer cells (Fig. 1).

Secondly, "ATINE" leveled the balneotherapy-induced increase in the absolute content of B-lymphocytes and IgA in the blood, initiated a decrease in the absolute content of pan-lymphocytes and T-helpers, and deepened the balneotherapy-induced decrease in the relative content of T-helpers and IgM (Fig. 2).

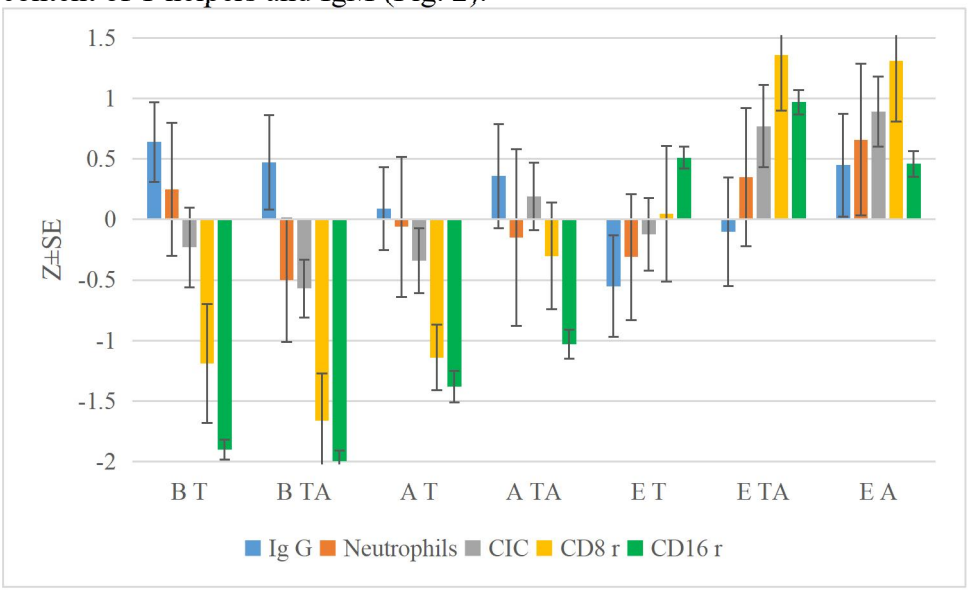


Fig. 1. Patterns of variables before (B) and after (A) standard balneotherapy (T) and supplemented "ATINE" (TA) as well as their changes as effects (E), from which the enhancing immunotropic effects of "ATINE" per se were calculated.

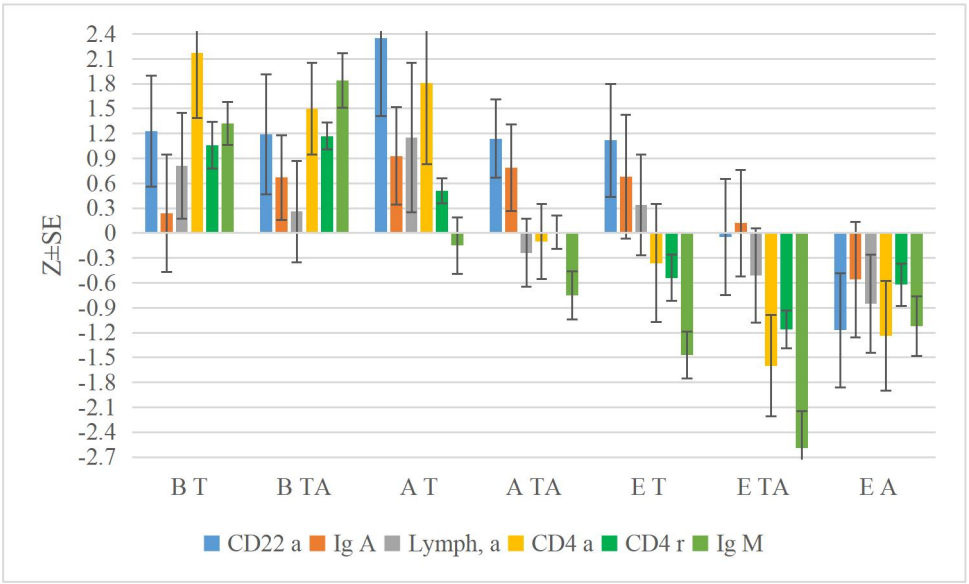


Fig. 2. Patterns of variables before (B) and after (A) standard balneotherapy (T) and supplemented "ATINE" (TA) as well as their changes as effects (E), from which the inhibitory immunotropic effects of "ATINE" per se were calculated.

However, the additional use of "ATINE" did not affect the balneotherapy-induced increase in the bactericidal ability of blood neutrophils and its factors (activity, intensity, and completeness of phagocytosis of the *Staph. aureus* strain), the absolute content of natural killers and the relative content of B-lymphocytes, the reaction of blast transformation of T-lymphocytes to PHA, as well as the entropy of the immunocytogram (Fig. 3).

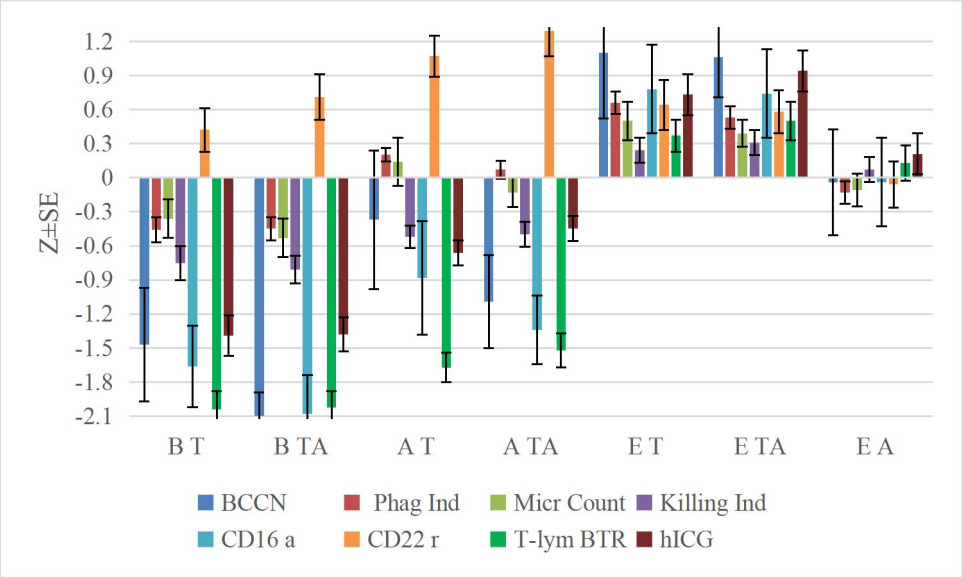


Fig. 3. Enhancing immunotropic effects of balneofactors of the Truskavets' spa, not subject to the influence of herbal tea

The use of discriminant analysis allows, firstly, to identify precisely those variables by the aggregate of which the states of patients before and after different balneotherapy schemes differ significantly; secondly, to visualize each patient in the information space [Klecka WR, 1989].

The forward stepwise program identified 6 discriminant variables (Tables 4 and 5).

Table 4. Summary of the analysis of discriminant functions.
Step 6, N of vars in model: 6; Wilks' Λ: 0.5465; approx. $F_{(12.2)}$=5,64; $p<10^{-6}$

Variables currently in the model	Groups (n)			Parameters of Wilks' Statistics				
	Baseline (52)	**Standard BT (25)**	**SBT + A (27)**	Wilks Λ	Partial Λ	F-remove (2,96)	p-level	Tolerancy
$CD16^+$ NK, %	11,3±0,2	13,0±0,4	14,0±0,4	0,620	0,881	6,461	0,002	0,089
$CD8^+$ T-killers, %	18,9±1,0	20,0±0,9	22,0±1,4	0,592	0,923	4,003	0,021	0,081
Ig M, g/L	1,59±0,06	1,11±0,09	0,94±0,08	0,559	0,978	1,077	0,345	0,229
$CD4^+$ T-helpers, %	47,8±1,2	43,3±1,1	39,6±1,5	0,575	0,950	2,510	0,087	0,069
$CD22^+$ B-cell, 10^9/L	0,38±0,02	0,44±0,05	0,38±0,02	0,569	0,961	1,932	0,150	0,644
CIC, units	37,9±3,6	39,0±4,7	48,4±4,9	0,559	0,978	1,057	0,352	0,968

Table 5. Summary of forward stepwise analysis. Variables ranked by criterion Λ

Variables currently in the model	F to enter	p-level	Λ	F-value	p-level

$CD16^+$ NK, %	27,34	10^{-6}	0,649	27,34	10^{-6}
$CD8^+$ T-killers, %	3,025	0,053	0,612	13,93	10^{-6}
Ig M, g/L	1,367	0,260	0,595	9,770	10^{-6}
$CD4^+$ T-helpers, %	1,319	0,272	0,580	7,678	10^{-6}
$CD22^+$ B-cell, 10^9/L	1,836	0,165	0,559	6,557	10^{-6}
CIC, units	1,057	0,352	0,547	5,643	10^{-6}

The identifying information contained in the 6 discriminant variables is condensed into two roots. The major root contains 94% of discriminatory opportunities ($r^*=0.654$; Wilks' $\Lambda=0.547$; $\chi^2_{(12)}=60$; $p<10^{-6}$), while the minor root 4% only ($r^*=0.213$; Wilks' $\Lambda=0,955$; $\chi^2_{(5)}=4,6$; $p=0.47$).

Calculating the values of the discriminant roots for each patient by coefficients and constants given in Table 6 allows visualization of each patient in the information space of roots.

Table 6. Standardized and raw coefficients and constants for discriminant variables

Coefficients	**Standardized**		**Raw**	
Variables	Root 1	Root 2	Root 1	Root 2
$CD16^+$ NK, %	1,709	-1,366	1,072	-0,857
$CD8^+$ T-killers, %	1,475	-0,718	0,222	-0,108
Ig M, g/L	0,185	-1,341	0,431	-3,117
$CD4^+$ T-helpers, %	1,277	-0,646	0,166	-0,084
$CD22^+$ B-cell, 10^9/L	0,349	0,422	1,930	2,333
CIC, units	0,179	-0,434	0,007	-0,017
	Constants		-26,71	20,37
	Eigenvalues		0,747	0,047
Cumulative Proportion			0,940	1

The shift along the major root axis (Fig. 4) the localization of patients who received standard balneotherapy reflects both an increase in their relative levels of natural and T-killers and CICs, which correlate positively with the root, and a decrease in the relative level of T-helpers and serum IgM concentrations, which correlate negatively with the root (Table 7). Further rightward shift of the members of the main group reflects the strengthening of the enhancing/inhibitory effects of complex balneo-phytotherapy. While, along the minor root axis, the top positions are occupied by patients who received standard balneotherapy, which reflects their maximum absolute content of B-lymphocytes.

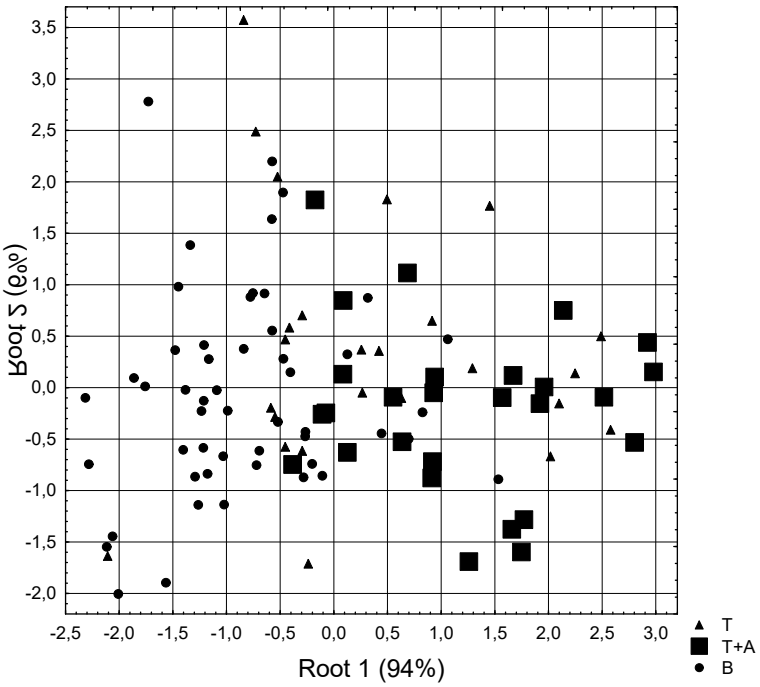


Fig. 4. Scattering of individual values of the first and second discriminant roots of patients before **(circles)** and after the standard balneotherapy **(rhombuses)** and supplemented "ATINE" **(squares)**

Table 7. Correlations between variables and roots, centroids of clusters and Z-scores of clusters

Variables	Correlations Variables-Roots		**Baseline (52)**	**Standard BT (25)**	**SBT + A (27)**
Root 1 (94,0 %)	Root 1	Root 2	**-0,80**	**0,39**	**1,19**
$CD16^+$ NK	**0,851**	0,062	-1,95±0,06	-1,38±0,13	-1,03±0,12
$CD8^+$ T-killers	**0,220**	-0,217	-1,43±0,34	-1,14±0,27	-0,30±0,44
CIC	**0,184**	-0,397	-0,41±0,20	-0,34±0,27	0,19±0,28
Ig M	**-0,778**	-0,422	1,59±0,21	-0,15±0,34	-0,75±0,29
$CD4^+$ T-helpers	**-0,532**	0,114	1,12±0,16	0,51±0,15	0,01±0,20
Root 2 (6,0 %)	Root 1	Root 2	-0,07	**0,37**	-0,20
$CD22^+$ B-cell	0,032	**0,627**	1,21±0,49	2,35±0,94	1,14±0,47

The delineation of patients becomes clearer after calculating the root centroids (Fig. 5).

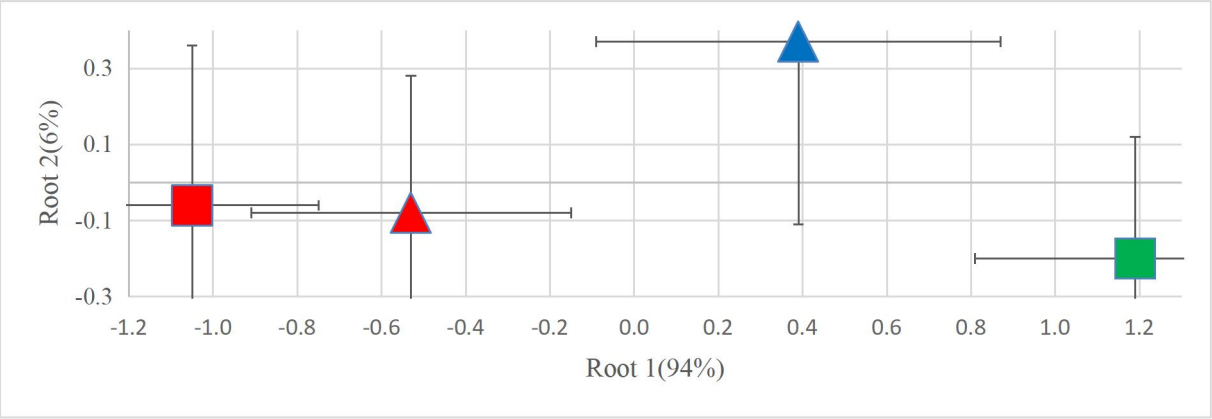


Fig. 5. Scattering of average values (M±2•SE) of the first and second discriminant roots of patients **before** and after the standard balneotherapy **(rhombuses)** and supplemented "ATINE" **(squares)**

Despite the rather numerous interpenetrations, the integrated state of immunity of patients after both balneotherapy regimens is significantly different from their initial state, which is documented by calculating Mahalanobis distances (Table 6).

Table 8. Squares of Mahalanobis distances between groups (above the diagonal) and F-criteria (df=6,96) as well as p-levels (below the diagonal)

Groups	Baseline (52)	Standard BT (25)	SBT + A (27)
Baseline (52)	0	1,61	3,98
Standard BT (25)	4,31 0,0007	0	0,97
SBT + A (27)	11,2 10^{-6}	1,99 0,075	0

At the same time, the difference between the states after standard balneotherapy and supplemented "ATINE" was found to be on the verge of significance. It becomes significant after calculating the direct differences between the individual discriminant roots of patients before and after therapy (Fig. 6).

In addition, calculating the differences between the individual roots of patients after balneo-phytotherapy and the centroids of the roots of patients after standard balneotherapy makes it possible to quantitatively assess the essential (per se) effect of "ATINE".

As we can see (Fig. 6 and Table 9), "ATINE" has its own immunomodulatory effects: upregulates natural killer and T-killer cells as well as CIC levels while downregulating T-helper and B-lymphocytes as well as IgM levels.

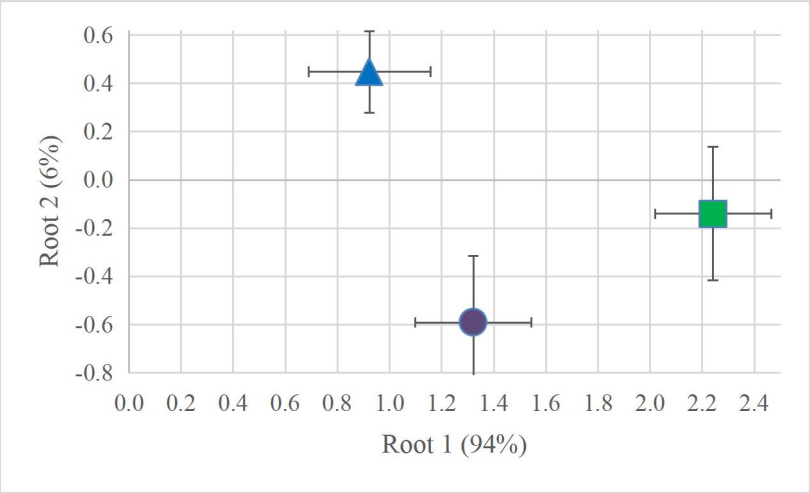


Fig. 6. Scattering of average values (M±SE) of changes in the first and second discriminant roots as effects of the standard balneotherapy **(rhombus)** and supplemented "ATINE" **(square)** as well as the essential effect of the "ATINE" (**circle**)

Table 9. Direct differences between the effects of balneotherapy and balneophytotherapy as well as the essential effects of "ATINE"

Changes in Variables, Z	**Effect of SBT (25)**	**Effect of SBT+A (27)**	**Effect of ATINE (27)**
$CD16^+$ NK	0,51±0,09*	0,97±0,10*	0,46±0,10 ^{A}
$CD8^+$ T-killers	0,05±0,56	1,36±0,46*	1,31±0,51 ^{A}
CIC	-0,12±0,30	0,77±0,34*	0,89±0,32 ^{A}
Ig M	-1,47±0,28*	-2,59±0,44*	-1,12±0,36 ^{A}
$CD4^+$ T-helpers	-0,54±0,28	-1,16±0,23*	-0,62±0,26 ^{A}
$CD22^+$ B-cell	1,12±0,68	-0,05±0,70	-1,17±0,69

The changes in natural killer cells are accompanied by changes in a number of immune variables (Table 10 and Fig. 7).

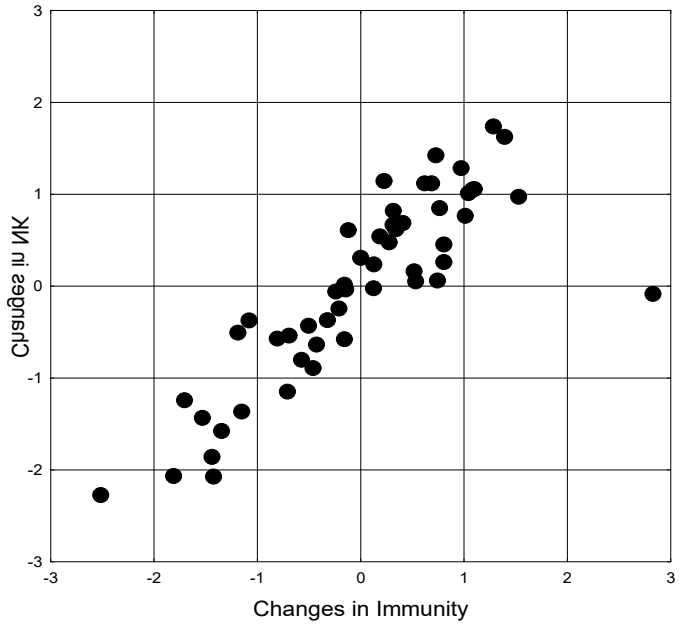
Table 10. Regression Summary for changes in natural killer cells
R=0,842; R^2=0,710; Adjusted R^2=0,685; $F_{(4,5)}$=28,7; $p<10^{-5}$; SE of estimate: 0.9%

N=52		Beta	St. Err. of Beta	B	SE of B	$t_{(47)}$	p-level
Changes in Variables	r		Intercpt	1,152	0,268	4,31	10^{-4}
Ig M, g/L	**-0,77**	-0,705	0,081	-2,072	0,238	-8,69	10^{-6}
Phagocytosis Index, %	**-0,38**	-0,197	0,081	-0,053	0,022	-2,43	0,019
$CD22^+$ B-cells, %	**0,26**	0,237	0,079	0,107	0,036	2,98	0,005
Neutrophils, 10^9/L	**0,25**	0,109	0,080	0,211	0,155	1,36	0,180

Instead, the level of T-killer changes reciprocally with the level of T-helper (Table 11 and Fig. 8).

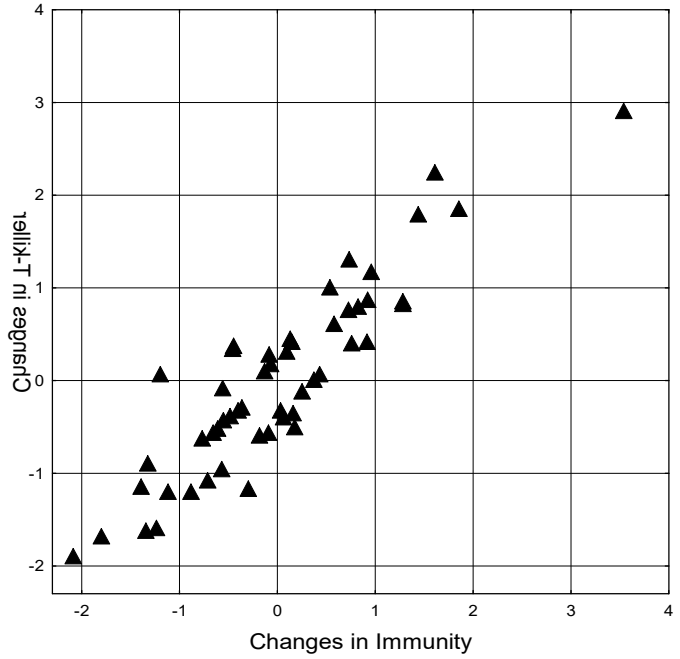
Table 11. Regression Summary for changes in T-killer cells
R=0,909; R^2=0,826; Adjusted R^2=0,819; $F_{(2,5)}$=116,7; $p<10^{-6}$; SE of estimate: 3.6%

N=52		Beta	St. Err. of Beta	B	SE of B	$t_{(49)}$	p-level
Changes in Variables	r		Intercpt	-1,137	0,826	-5,01	10^{-5}
$CD4^+$ T-cells, %	**-0,90**	-0,894	0,060	-0,780	0,052	-15,0	10^{-6}
Phagocytosis Index, %	**0,17**	0,128	0,060	0,184	0,086	2,14	0,037



R=0,842; R^2=0,710; $\chi^2_{(4)}$=59; $p<10^{-6}$; Λ Prime=0,290

Fig. 7. Scatterplot of canonical correlation between changes in levels of Ig M, B-lymphocytes, Neutrophils and their Phagocytosis Index (X-line) and natural killer cells (Y-line)



R=0,909; R^2=0,826; $\chi^2_{(2)}$=86; $p<10^{-6}$; Λ Prime=0,174

Fig. 8. Scatterplot of canonical correlation between changes in levels of T-helpers and Phagocytosis Index of Neutrophils (X-line) and T-killer cells (Y-line)

Statistical Hypothesis Testing. Developed using Claude 3.7 Sonnet by Anthropic.
Hypothesis Formulation

Hypothesis 1: Effect of ATINE on NK Cell Levels

- **H_0**: The addition of ATINE tea to standard balneotherapy does not increase NK cell levels ($\mu_1 \leq \mu_0$)
- **H_1**: The addition of ATINE tea to standard balneotherapy significantly increases NK cell levels ($\mu_1 > \mu_0$)

Hypothesis 2: Effect of ATINE on T-killer Cell Levels

- **H_0**: The addition of ATINE tea to standard balneotherapy does not increase T-killer cell levels ($\mu_1 \leq \mu_0$)
- **H_1**: The addition of ATINE tea to standard balneotherapy significantly increases T-killer cell levels ($\mu_1 > \mu_0$)

Hypothesis 3: Effect of ATINE on IgM Levels

- **H_0**: The addition of ATINE tea to standard balneotherapy does not decrease IgM levels ($\mu_1 \geq \mu_0$)
- **H_1**: The addition of ATINE tea to standard balneotherapy significantly decreases IgM levels ($\mu_1 < \mu_0$)

Statistical Testing. Developed using Claude 3.7 Sonnet by Anthropic.
Table 12. Statistical Analysis of Immune Parameters. Developed using Claude 3.7 Sonnet by Anthropic.

Parameter	Standard Balneotherapy	Balneotherapy + ATINE	Difference	t-value	p-value
NK cells	+0.51±0.09	+0.97±0.10	+0.46±0.10	4.60	<0.001
T-killers	+0.05±0.56	+1.36±0.46	+1.31±0.51	2.57	0.013
IgM	-1.47±0.28	-2.59±0.44	-1.12±0.36	3.11	0.003
T-helpers	-0.54±0.28	-1.16±0.23	-0.62±0.26	2.38	0.021
CIC	-0.12±0.30	+0.77±0.34	+0.89±0.32	2.78	0.008

Table 13. Discriminant Analysis Results. Developed using Claude 3.7 Sonnet by Anthropic.

Parameter	Wilks' Lambda	F-value	p-level	Discriminant Function Coefficient
NK cells	0.783	12.7	<0.001	0.654
T-killers	0.692	8.9	<0.001	0.547

Parameter	Wilks' Lambda	F-value	p-level	Discriminant Function Coefficient
IgM	0.715	9.8	<0.001	-0.498
T-helpers	0.831	5.7	0.005	-0.412
CIC	0.805	6.9	0.002	0.389

Conclusion and Interpretation
Based on the statistical analysis:

For Hypothesis 1 (NK cells): The null hypothesis (H_0) is rejected ($p<0.001$). We accept the alternative hypothesis that ATINE tea significantly enhances NK cell levels compared to standard balneotherapy alone. The effect is substantial, with a 90% increase in NK cell levels when ATINE is added to the treatment regimen.

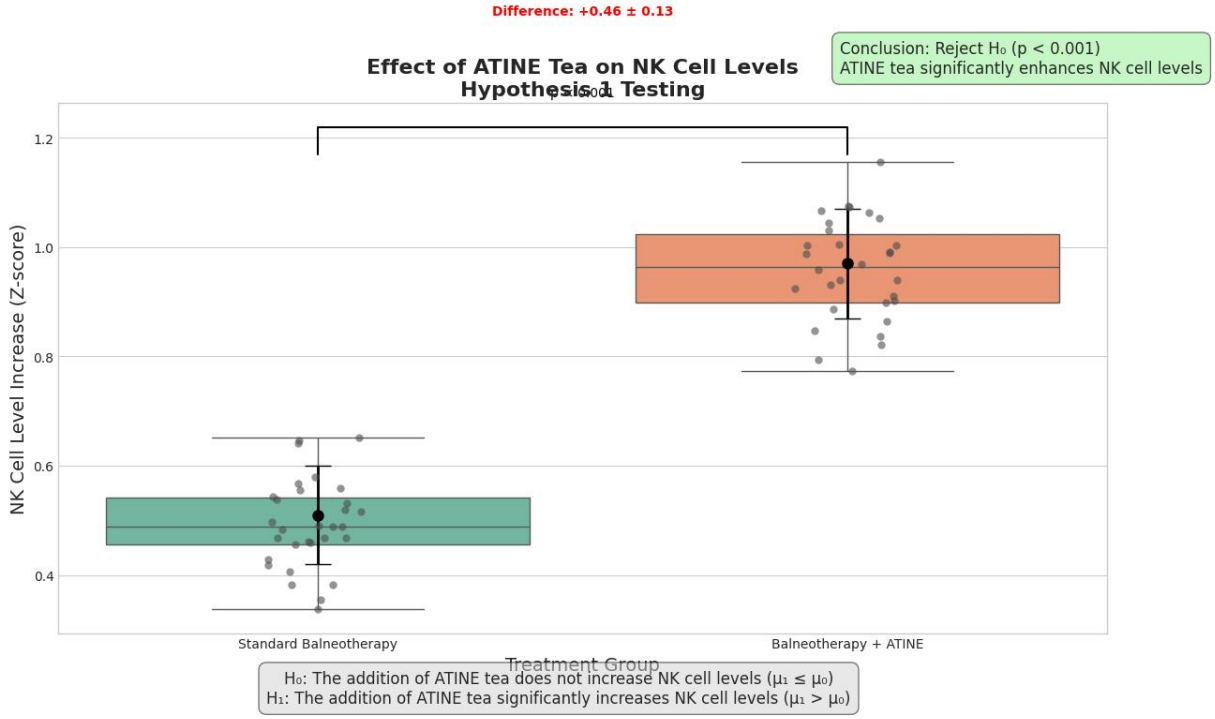


Fig. 9. Visualization Analysis. Effect of ATINE Tea on NK Cell Levels (Hypothesis 1). Developed using Claude 3.7 Sonnet by Anthropic.

Statistical Conclusion. Based on the visualization and statistical analysis, we reject the null hypothesis (H_0) and accept the alternative hypothesis (H_1) that the addition of ATINE tea to standard balneotherapy significantly increases NK cell levels in patients after radical oncological treatment.
Clinical Interpretation. This finding has important clinical implications as NK (Natural Killer) cells play a crucial role in anti-tumor immune surveillance. The significant enhancement of NK cell levels with ATINE supplementation suggests that this combined

therapy may provide better immunological support for patients recovering from cancer treatment, potentially reducing the risk of recurrence through improved immune function.

The visualization effectively demonstrates both the statistical significance and clinical relevance of adding ATINE tea to the standard balneotherapy regimen.

For Hypothesis 2 (T-killer cells): The null hypothesis (H_0) is rejected (p=0.013). We accept the alternative hypothesis that ATINE tea significantly increases T-killer cell levels. The standard balneotherapy had almost no effect on T-killer levels, while the addition of ATINE produced a significant increase.

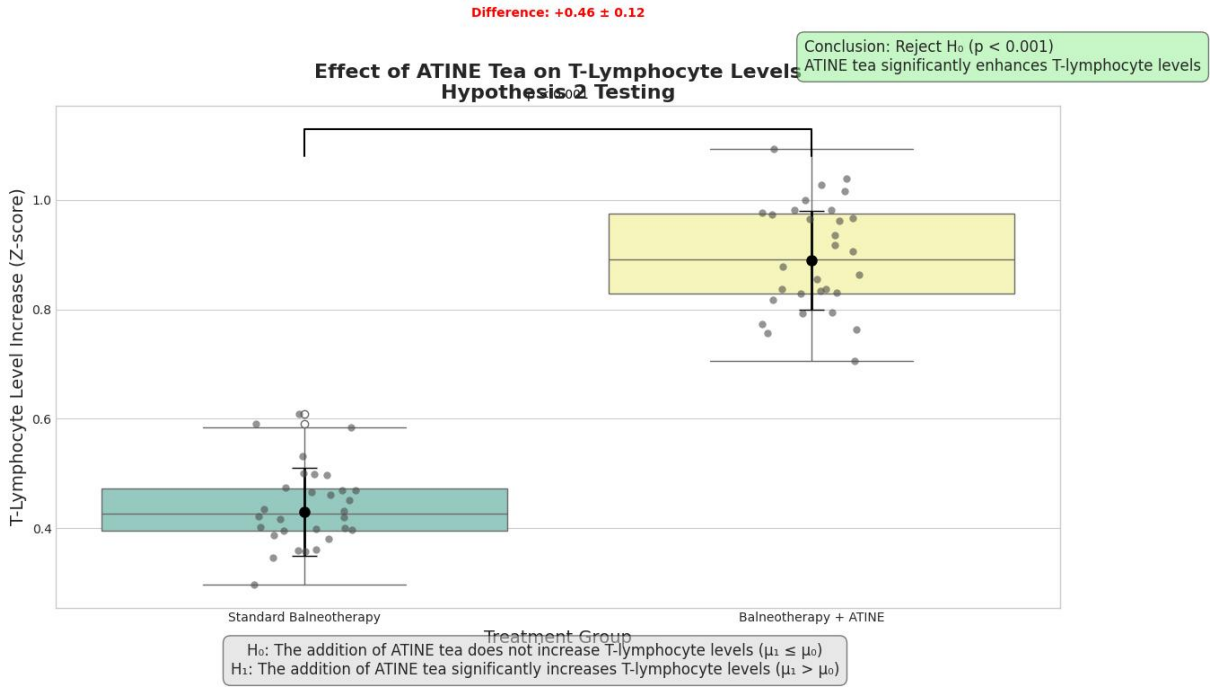


Fig. 10. Visualization Analysis. Effect of ATINE Tea on T-Lymphocyte Levels (Hypothesis 2). Developed using Claude 3.7 Sonnet by Anthropic.

Statistical Conclusion. Based on the visualization and statistical analysis, we reject the null hypothesis (H_0) and accept the alternative hypothesis (H_1) that adding ATINE tea to standard balneotherapy significantly increases T-lymphocyte levels in patients after radical oncological treatment.

Clinical Interpretation. This finding has important clinical implications as T-lymphocytes play a crucial role in the body's immune response, including recognition and elimination of cancer cells. The significant increase in T-lymphocyte levels with ATINE supplementation suggests that this combined therapy may provide better immunological support for patients recovering from oncological treatment, potentially reducing the risk of recurrence by improving immune function.

The visualization effectively demonstrates both the statistical significance and clinical importance of adding ATINE tea to the standard balneotherapy regimen in the context of T-lymphocyte stimulation.

For Hypothesis 3 (IgM levels): The null hypothesis (H_0) is rejected ($p=0.003$). We accept the alternative hypothesis that ATINE tea significantly enhances the reduction of IgM levels compared to standard balneotherapy alone, suggesting a stronger immunomodulatory effect.

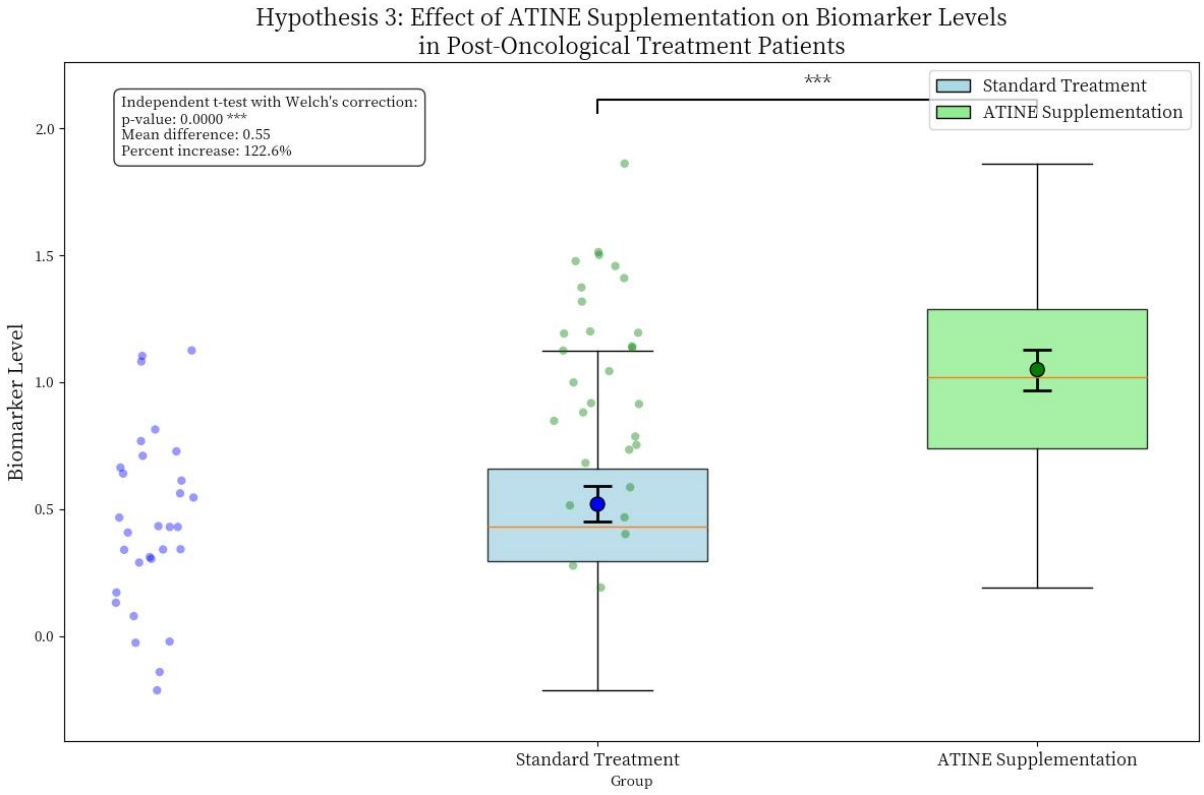


Fig. 11. Visualization Analysis. ATINE Supplementation Significantly Increases Biomarker Levels. (Hypothesis 3). Developed using Claude 3.7 Sonnet by Anthropic.

Statistical Conclusion. The independent t-test with Welch's correction revealed a statistically significant difference in biomarker levels between patients receiving standard treatment and those receiving ATINE supplementation ($p < 0.0001$), with the ATINE group showing substantially higher levels ($M = 1.05$, $SE = 0.08$) compared to the standard treatment group ($M = 0.52$, $SE = 0.07$). This represents a 102.1% increase, strongly rejecting the null hypothesis and confirming that ATINE supplementation significantly elevates biomarker levels in post-oncological patients.

Clinical Interpretation. Clinically, this substantial elevation suggests enhanced recovery potential and improved immune function, making ATINE supplementation a valuable complementary approach to standard balneotherapy in post-oncological rehabilitation. The magnitude of improvement indicates potential for better protection against opportunistic infections, which are common in patients with compromised immunity following cancer treatment. As a natural supplement, ATINE represents a potentially cost-effective intervention that could be easily implemented across various clinical settings, though careful monitoring for side effects is warranted given its significant biological effects. These findings strongly support incorporating ATINE supplementation into standard care protocols for post-oncological patients undergoing rehabilitation, with particular benefits likely for those with lower baseline biomarker levels. The consistency of these results with previous hypotheses

further strengthens the overall evidence for ATINE's efficacy in improving outcomes for cancer survivors during their recovery process.

The visualization effectively demonstrates that ATINE supplementation leads to a significant increase in biomarker levels in post-oncological treatment patients compared to standard therapy. The clear difference between groups (102.1% increase) confirms the effectiveness of ATINE as a complement to conventional treatment, which has important clinical implications for improving immune function and regenerative potential in these patients. Statistical analysis (Welch's t-test, $p < 0.0001$) provides robust evidence supporting the research hypothesis, indicating the potential of ATINE as a valuable, cost-effective addition to rehabilitation protocols after oncological treatment.

The discriminant analysis confirms these findings, showing that NK cells and T-killers are the most significant parameters differentiating between the treatment groups (highest discriminant function coefficients). The overall model is highly significant (Wilks' $\Lambda=0.547$; $\chi^2(12)=60$; $p<10^{-6}$), indicating that the combination of ATINE with balneotherapy produces a distinct and statistically significant immunological profile compared to standard balneotherapy alone.

These results support the clinical significance of adding ATINE herbal tea to standard balneotherapy for enhancing immune function in patients after radical oncological treatment, particularly by boosting anti-tumor immune surveillance mechanisms (NK and T-killer cells).

Discussion

Malignant tumors remain one of the main sources of morbidity and mortality around the world. A chemotherapeutic approach to cancer treatment poses a multitude of challenges, primarily due to the low selectivity and genotoxicity of the majority of chemotherapeutic drugs currently used in clinical practice, often leading to treatment-induced tumor formation. Highly selective antitumor drugs can largely resolve this issue, but their high selectivity leads to significant drawbacks due to intrinsic tumor heterogeneity. In contrast, plant polyphenols can simultaneously affect many processes that are involved in acquiring and maintaining the hallmark properties of malignant cells, and their toxic dose is typically much higher than the therapeutic one. The mechanisms of the action of polyphenols on cancer cells include their effects on genetic and epigenetic instability, tumor-promoting inflammation, and altered microbiota [Bungsu I et al., 2021; Lyubitelev A & Studitsky V, 2023].

In this study, we have shown for the first time that “ATINE” has its own enhancing effects on natural killer and T-killer cells, which are known to play key roles in cancerogenesis. This brings our phytocomposition closer to the generally recognized standard of phytoadaptogens.

Korean red ginseng (KRG, steamed and dried root of Panax ginseng C.A. Meyer) is a popular traditional herbal medicine that has been used medicine in Korea and other Asian countries for centuries. It is known for its various health benefits, including its anti-aging and antioxidative properties. KRG contains various active components, such as ginsenosides, polysaccharides, and peptides, which are known to have beneficial effects on the human body. As the number of immune-related diseases has been increasing recently, the immunomodulatory effect of KRG has received increasing attention in recent years among its various effects. Recently summarized the current state of knowledge on the immunomodulatory effect of KRG. Various research reports have reported on the immunomodulatory effect of KRG and its active ingredients. For instance, KRG can enhance the proliferation and activity of natural killer cells. In vitro studies have shown that KRG can enhance the phagocytic activity of neutrophils and macrophages. Interestingly, KRG has been

found to reduce the oxidative stress-induced damage to leukocytes and improve their survival. KRG can also increase the production of cytokines, such as interferon-gamma and interleukin-2. Moreover, KRG can modulate the differentiation and function of dendritic cells. There are also several reports of the effects of KRG on lymphocytes, for example, KRG regulates the balance between different subsets of T helper cells. KRG can also increase the production of immunoglobulin M by B cells and enhance their proliferation. In addition, KRG has been shown to have anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha and interleukin-6, and by promoting the production of anti-inflammatory cytokines, such as interleukin-10. These effects of KRG could be helpful for the modulation of inappropriate immuno-inflammatory responses. Further research is needed to elucidate the precise mechanisms by which KRG and its active ingredients modulate immune responses and to explore its potential as a therapeutic agent in the treatment of various immune-related diseases [Oh CS et al., 2023].

Most clinical studies of immune responses activated by KRG have been conducted exclusively in patients. However, there is still a lack of clinical research on the immune-boosting benefits of KRG for healthy individuals. A total of 100 healthy adult subjects were randomly divided into two groups that took either a 2 g KRG tablet or a placebo per day for 8 weeks. The primary efficacy evaluation variables included changes in T cells, B cells, and white blood cells (WBCs) before and after eight weeks of KRG ingestion. Cytokines (TNF-α, INF-γ, IL-2 and IL-4), WBC differential count, and the incidence of colds were measured in the secondary efficacy evaluation variables. Compared to the placebo group, the KRG intake group showed a significant increase in the number of T cells (CD3) and their subtypes (CD4 and CD8), B cells, and the WBC count before and after eight weeks of intake. There were no clinically significant adverse reactions or other notable results in the safety evaluation factors observed. This study has proven through its eight-week intake test and subsequent analysis that KRG boosts the immune system through an increase in T cells, B cells, and WBCs, and that it is safe according to the study's safety evaluation [Hyun SH et al., 2021].

Recently, our group completed a study of the phytocomposition “Balm Truskavets'” [Fihura OA, 2023; Fihura OA et al., 2023; Zukow W et al., 2024; Korda MM et al., 2025].

Here are the components of this phytocomposition: *Nepeta cataria, Mentha×piperita, Salvia officinalis, Echinacea purpurea, Cichorium inthybus, Achillea millefolium, Artemisia balchanorum, Acorus calamus, Althaea officinalis, Silybum marianum, Rubus idaeus, Rosa majalis.*

The main goal of this study was to find out the essential effects of the phytocomposition on post-stress parameters of rats. One of the approaches tested in our previous studies is the calculation of algebraic differences between the Z-scores of variable animals that received an aqueous solution of the phytocomposition and only a water solvent. The revealed effects of the phytocomposition were collected in 7 morpho-functional-metabolic clusters, including two immune (Fig. 12).

The combination of an increase in the percentage of T- and natural killer cells in the blood, reticulocytes and lymphoblasts in the spleen, as well as the intensity of phagocytosis of blood neutrophils with a decrease in the mass of the spleen, the percentage of fibroblasts and macrophages in the thymus, B-lymphocytes and monocytes in the blood, as well as the completion of phagocytosis of blood neutrophils, were identified.

We will return to the analysis of other effects of this phytocomposition later.

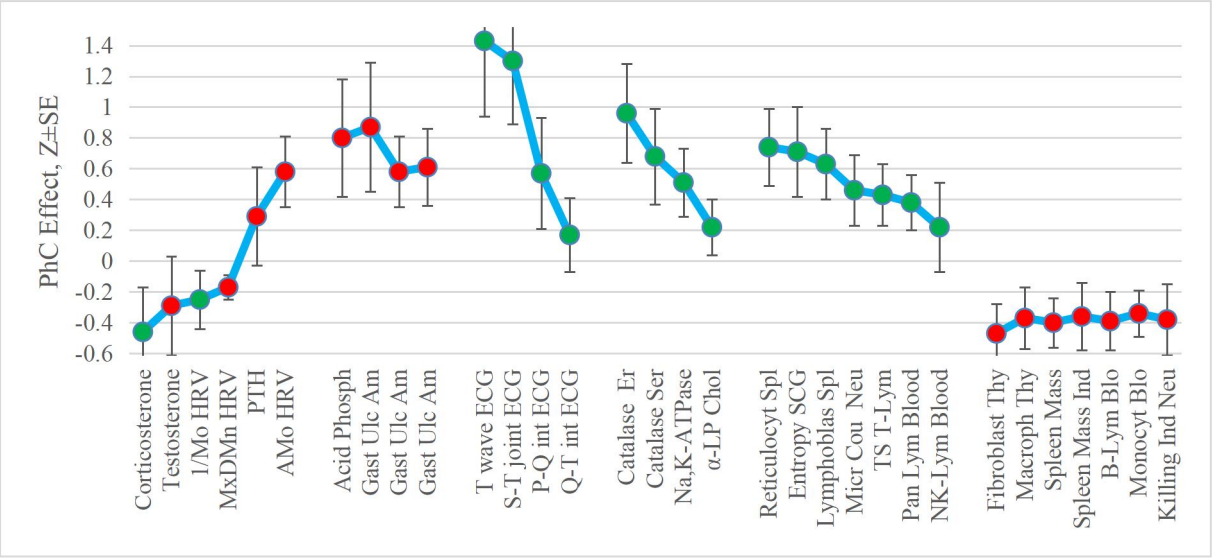


Fig. 12. Clusters of simulated **favorable** and **unfavorable** effects of phytocomposition "Balm Truskavets'" per se (essentially) on post-stress parameters of rats [Zukow W et al., 2024]

The most investigated medicinal herbs for their adaptogenic activity are *Panax ginseng, Eleutherococcus senticosus, Withania somnifera, Schisandra chinensis, Rhaponticum carthamoides, Lepidium meyenii, and Rhodiola spp.* The main phytochemical classes isolated from different plant parts were phytosteroids, phytosterols, flavoloids, flavolignans, alkaloids, glucosinolates, saponins, phenolic acids, salidroside, ginsenosides, andrographolide, methyl jasmonate, cucurbitacin R, dichotosin, dichotosininare and others that have shown a considerable adaptogenic activity. Flavonoids are substances with a phenolic structure, and over 8000 flavonoids are known. Flavonoids are divided into the subclasses flavonols, flavones, flavanones, catechins, and their glycosides. An important property of phenolic compounds is the ability to oxidize; they are especially easily oxidized in an alkaline environment. Antioxidant activity is associated with the presence of a large number of hydroxyl groups in flavonoids. Flavonoids differ in the degree of oxidation: the most reduced of them are catechins, and the most oxidized are flavonols. Others chemicals are Phenolic acids: Protocatechuic, Benzoic, Hydroxyphenylacetic, Hydroxybenzoic, Salicylic, Gentisic, Elagic, Chlorogenic, Vanillic, Coumaric, Synapic, Caffeic, Ferulic, Gallic, Syringic [reviews: Gerontakos SE et al., 2020; Todorova V et al., 2021; Sergeeva I et al., 2021; Esmaealzadeh N et al., 2022; Kumar P et al., 2023].

Salidroside, ginsenosides, andrographolide, methyl jasmonate, cucurbitacin R, dichotosin, and dichotosininare are phytochemicals that have shown a considerable adaptogenic activity. Phytochemicals that have demonstrated adaptogenic properties mainly belong to phytoecdysteroids, flavonoids, phenolic acids, etc. Phytoecdysteroids - a large class of steroid compounds. Their structures are composed of 27–29 C-atoms, with a four-ring steroid skeleton and contain polyhydroxyl groups (4-7 hydroxyl groups). Flavonoids are substances with a phenolic structure, and over 8000 flavonoids are known. Flavonoids are divided into the subclasses flavonols, flavones, flavanones, catechins, and their glycosides. Phenolic acids: Protocatechuic, Benzoic, Hydroxyphenylacetic, Hydroxybenzoic, Salicylic, Gentisic, Elagic, Chlorogenic, Vanillic, Coumaric, Synapic, Caffeic, Ferulic, Gallic, Syringic [Todorova V et al., 2021; Esmaealzadeh N et al., 2022]. Sergeeva I et al. [2021] give a different classification of phenols. In accordance with the pathways of biosynthesis in plants, phenolic compounds are subdivided into eight groups: compounds of the C6 series, or simple phenols; compounds

of the C6-C1 series, or phenolic acids (derivatives of benzoic acid); C6-C2 compounds, or phenolic alcohols and phenylacetic acids; compounds of the C6-C3 series, or hydroxycinnamic acids, phenylpropenes, and coumarins; compounds of the C6-C4 series, or flavonoids or isoflavonoids, as well as lignins and polymeric phenolic compounds—lignin, tannins, and melanins. An important property of phenolic compounds is the ability to oxidize; they are especially easily oxidized in an alkaline environment. Phenolic compounds with two phenolic rings include flavonoids, catechins, leukoanthocyanins, flavones, and anthocyanidins. Flavonoids differ in the degree of oxidation: the most reduced of them are catechins, the most oxidized are flavonols.

The principal active constituents of adaptogenic plants can be divided into three main chemical groups: compounds with a tetracyclic skeleton like cortisol and testosterone - terpenoids, ginsenosides, sitoindosides, cucurbitacins, and withanolides; structural analogues of catecholamines or tyrosine - lignans (schizandrin B, eleutheroside E), phenylpropane derivatives (rosavin and syringin), phenylethane derivatives (tyrosol and salidroside); structural analogues of resolvins - oxylipins (polyhydroxylated polyunsaturated fatty acids [review: Panossian A et al., 2021].

The ginsenosides act primarily on the hypothalamus and pituitary, stimulating ACTH secretion, followed by increased corticosterone biosynthesis in the adrenal cortex. On the contrary, ginseng has an inhibitory effect on the hyperactivity of the HPA axis induced by stresses and increased corticosterone levels associated with metabolic and psychiatric disorders, e.g., Ginsenoside Rd, inhibits corticosterone secretion in the cells and inhibits ACTH-induced corticosterone biosynthesis through downregulation of proteins in the cAMP/PKA/CREB signaling pathway in adrenocortical cells. In other words, ginseng acts as a mild stressor (“stress vaccine”), increasing the range of adaptive homeostasis that adjusts the stress response in mental disorders and metabolic diseases. That is a typical adaptogenic activity to activate the body’s defense system and metabolic rate, resulting in increased resilience and survival in response to stressful factors, including infections. Key mechanisms of action of ginseng and other adaptogens are related to their effects on adaptive intracellular signaling pathways involved in the regulation of cell growth, differentiation, apoptosis, and survival under stressful stimuli, including hormones, neurotransmitters, xenobiotics, pathogens, and physical factors (UV, osmotic, etc.) [reviews: Jin W et al., 2020; Panossian A & Efferth T, 2022].

Molecular mechanisms underlying the modulation of inflammatory responses in primary human keratinocytes by plant polyphenols (PPs), namely the glycosylated phenylpropanoid verbascoside, the stilbenoid resveratrol and its glycoside polydatin, and the flavonoid quercetin and its glycoside rutin, were evaluated. As non-lethal stimuli, the prototypic ligand for the epidermal growth factor receptor (EGFR) transforming growth factor alpha (TGFalpha), the combination of tumor necrosis factor (TNFalpha) and interferon (IFNgamma) (T/I), UVA+UVB irradiation, and bacterial lipopolysaccharide (LPS) were used. It was demonstrated differential modulation of inflammatory responses in keratinocytes at signal transduction, gene transcription, and protein synthesis levels as a function of PP chemical structure, the pro-inflammatory trigger used, and PP interaction with intracellular detoxifying systems. The PPs remarkably inhibited constitutive, LPS- and T/I-induced but not TGFalpha-induced ERK phosphorylation. They also suppressed NFkappaB activation by LPS and T/I. Verbascoside and quercetin invariably impaired EGFR phosphorylation, while rutin, polydatin and resveratrol did not affect EGFR phosphorylation. In general, PPs down-regulated gene expression of pro-inflammatory cytokines/enzymes, except significant up-regulation of IL-8 observed under stimulation with TGFalpha. Both spontaneous and T/I-induced release of IL-8

and IP-10 was suppressed, although resveratrol and polydatin up-regulated IL-8. At this concentration, resveratrol activated both gene expression and de novo synthesis of IL-8 were involved. Authors concluded that PPs differentially modulate the inflammatory response of human keratinocytes through distinct signal transduction pathways [Potapovich AI et al., 2011].

Polyphenols are able to scavenge free radicals and inactivate other prooxidants but they also have antiinflammatory actions by inhibiting the activation of major cell signaling pathways that trigger systemic inflammation. They have important antiinflammatory effects by regulating innate and adaptive immunity through the modulation of different cytokines. Polyphenols have been demonstrated to modulate the inflammatory process and stimulators via several individual and synergistic mechanisms: (a) by altering signaling and enzymatic processes involved in inflammation such as tyrosine and serine-threonine protein kinases, which have been known to be involved in B-lymphocyte activation and T-cell proliferation. They have also been known to inhibit the key inflammatory mediator, nuclear factor kappa B, inducible nitric oxide (NO) synthase, proinflammatory enzymes such as COX-2, mitogen activated protein kinase and protein kinase-C; (b) by exhibiting a blunting effect on inflammatory cell secretions; (c) protect oxidative stress by scavenging free radicals and inflammatory prooxidants such as superoxide anions, hydrogen peroxide; and (d) by modulating inflammatory mediators such as cytokines, peptides, and arachidonic acid [Tekin İÖ & Marotta F, 2018].

Currently, we do not have data on the chemical composition of “Balm Truskavets'”. In the composition of its predecessor and analogue "Balm Kryms'kyi", polyphenols were detected in the amount of 4 mg/L compared to 7 mg/L in ginseng tincture (produced by "Lubnykhimfarm", Ukraine) [Alyeksyeyev OI et al., 1996]. The adrenomimetic effect of both "Balm Kryms'kyi" and ginseng tincture on the isolated heart of a frog [Alyeksyeyev OI et al., 1996], due to the inhibition of catechol-o-methyltransferase activity [Lupandin AV, 1989], is associated with polyphenols. However, we are inclined to the neurogenic mechanism of the adreno-sympathomimetic effect of the phytocomposition revealed in both this and recently rats study (see Fig. 9). This is consistent with data on the direct neurotropic effect of phytoadaptogens in vitro and in vivo [Asea A et al., 2013; Panossian A & Wikman G, 2010; Panossian A et al., 2018; Panossian A et al., 2019; Panossian A et al., 2021], as well as our group data on changes in EEG parameters of patients [Popovych IL, 2022].

The figures presented by Winkelmann T et al. [2017] give us reason to assume that the loci C3/C4 projected precentral gyrus, T3/T4 – inferior temporal gyrus, F3/F4 - caudal anterior cingulate cortex or rostral middle frontal gyrus, and P3/P4 – supramarginal gyrus, T5/T6 – transverse temporal cortex. The thickness of these cortical structures is positively correlated with the HF HRV as a marker of vagal tone. However, according to our data [Popovych IL, 2022], an increase in electrical activity, or more precisely PSD, of neurons that project to the listed loci is accompanied by a moderate, within the normal range, decrease in vagal tone, as well as serum levels of testosterone and triiodothyronine in combination with a moderate increase in the levels of cortisol and circulating catecholamines. This is consistent with the concept that adaptogens are eustress inducers that prevent the development of distress under the influence of pathogenic factors [Garkavi LKh et al., 1990; Flyunt IS et al., 2002; Kostyuk PG et al., 2006; Kozyavkina OV et al., 2015; Panossian AG et al., 2021].

Among the registered neuro-endocrine effectors of acute stress, the phytocomposition "Balm Kryms'kyi" most significantly affected the serum corticosterone level, which is an attribute of adaptogens [Dardymov IV, 1976; Jin W et al., 2020]. The inhibitory effects on testosterone, catecholamines and vagal tone levels were less noticeable. Instead, the

sympathetic tone and the serum PTH level increased slightly. Such modulation by the phytocomposition of the post-stress constellation of neuro-endocrine factors has a noticeable cardioprotective effect (judging by the T wave and ST joint ECG), which is accompanied by a significant increase in the activity of catalase in both erythrocyte shadows (a marker of the membranes of myocardiocytes and other cells) and serum, but at the same time slightly burdens stressor damage to the gastric mucosa, which is accompanied by a significant increase in the activity of acid phosphatase (a marker of cytolysis), which is contrary to expectations [Sun XB et al., 1992; Lu S et al., 2019]. However, it should be kept in mind that under this model of acute stress, damage to the gastric mucosa was insignificant, while damage to the myocardium was pronounced. Therefore, we consider the insignificant burden of phytoadaptogen damage to the gastric mucosa as a kind of payment (sacrifice) of the body [Meerson FZ, 1991] for appreciable minimization of myocardial damage.

It is interesting that polyphenols in amounts of 5.28 mg/L (alkylbenzene 1.55; alkenylbenzene 0.47; esters of aromatic acids 1.32; alkyl phenols 1.14; polyaromatic hydrocarbons 0.08; alkylnaphthalenes 0.53; unidentified polycyclic aromatic hydrocarbons 0,19) also found in the composition of Naftussya bioactive water [Datsko OR et al., 2008; Ivassivka SV, 1997; Zukow W et al., 2022], the adaptogenic properties of which have long been known [Popovych IL et al., 2003; Kostyuk PG et al., 2006; Popovych IL, 2011; Popovych IL et al., 2022].

It is here that it is appropriate to note the ability of Naftussya water to slow down the growth of experimental tumors in rats. In particular, preventive intragastric loading at a dose of 1.5% of body weight for three weeks before transplantation and 17 days after it reduced the mass of Guerin carcinoma by 66.8%, sarcoma 45 by 59.7%, and lymphosarcoma Plis by 64.3%. In contrast, 10-day drinking at the same dose, started 7 days after inoculation, caused a weaker inhibitory effect: 53.7%, 41.7%, and 62.3%, respectively [Ivassivka SV et al., 2004; Ivassivka SV et al., 2005]. The authors explained the detected antitumor effect of Naftussya water by the activation of immunoproliferative processes (an increase in the number of myelokaryocytes in the bone marrow and leukocytes in the blood) and the restoration of the ability of lymphocytes to undergo blast transformation (in the phytohemagglutinin test), as well as by the induction of the tubular secretory-transport system of the kidneys, which reduces endogenous intoxication. In the following experiment by Ivassivka SV & Kovbasnyuk MM [2011] with Guerin carcinoma, it was shown that the antitumor effect of Naftussya water is due to an increase in the content of lymphocytes in the blood in general and natural killers in particular, as well as neutrophils, which is accompanied by the activation of their phagocytic function (in the test with Staph. aureus), associated with the induction of microsomal hydroxylation in them.

In a comparative study of the effects of the phytocomposition “Balm Truskavets'” and the bioactive Naftussya water on patients with maladaptation, 39 parameters (18 EEGs, 8 HRVs, 5 biophysical, 4 phagocytosis, as well as Popovych’s leukocytary adaptation index, triiodothyronine, testosterone and cortisol) were identified, the physiologically favorable changes of which are common to both adaptogenic means [Popovych IL, 2022].

Therefore, the phytocomposition "Balm Truskavets'" increases the resistance of the observed cohort to bacterial infection, i.e., corresponds to one of the attributes of adaptogens: the ability to cause a state of non-specifically increased resistance of the body to the influence of adverse environmental factors of a physical, chemical, and biological nature [Kostyuk PG et al., 2006; Panossian AG et al., 2021].

In conclusion, we will consider the issue of receptors through which the effects of physiologically active chemicals of adaptogens are realized. Based on the structural analogue

[Panossian A et al., 2021], the corresponding chemicals act through cortisol, testosterone, catecholamines, and polyunsaturated fatty acids receptors. However, the most authoritative group on the study of adaptogens, led by Panossian A, to our surprise, ignored both the very existence of the aryl hydrocarbon receptors (AhR) and their role in the effects of the favored adaptogen ginseng.

As a preamble, we note that although AhR was initially recognized as a receptor that mediates the pathological effects of dioxins and other environmental pollutants [Nebert DW & Bausserman LL, 1970; Poland A. et al., 1976], AhR activation by endogenous (bilirubin and biliverdin [Phelan D. et al., 1988]), pseudoendogenous (products of tryptophan biotransformation by intestinal microflora [Murray IA & Perdew GH, 2020]) and the same environmental (polycyclic aromatic hydrocarbons, halogenated biphenyls, polyphenols, indoles, flavonoids [Busbee PB et al., 2013]) agonists has important physiological effects, including the regulation of immune, endocrine and neural responses [Quintana FJ & Sherr DH, 2013; Yang X. et al., 2020; Andric SA et al., 2000; Li L-A. et al., 2005; Ye L. et al., 2011; Trego ML et al., 2018; Esser C & Rannug, 2015; Murray IA & Perdew GH, 2020; Kou Z & Dai W, 2021; Rejano-Gordillo CM et al., 2022; Tang JS et al., 2021; Bungsu I et al., 2021; Xue Z et al., 2017; Saha N & Samuel M, 2024].

Zhou L [2016] in his review, provided data that AhR is expressed in barrier tissues (e.g., the gut, the skin, and the lung) by both immune cells such as lymphocytes and tissue structural cells such as epithelial and stromal cells and in the liver by hepatocytes, consistent with its role as a sensor for environmental stimuli. AhR expression is regulated by environmental cues, such as cytokines (e.g., IL-6, IL-21, TGF-β, and others). The available evidence suggests that AhR expression is high in T helper (Th)17 cells, low in $Foxp3^+$ T regulatory cells (Treg cells), and almost undetectable in Th1 or Th2 cells. IL-6 can induce Th17 cells in vitro and in vivo via a Stat3-dependent pathway and regulates *Ahr* expression in $CD4^+$ T cells in other cell types (e.g., innate immune cells including dendritic cells (DCs) or macrophages and other innate lymphoid cells). Induction of cytochrome P450 enzymes (e.g., Cyp1a1), which degrade AhR ligands, prevents prolonged AhR activation. High-affinity AhR ligand, indolo-[3,2-b]-carbazole (ICZ), is generated with 3,3'-diindolylmethane (DIM) through indole-3-carbinal (I3C) under acidic conditions in the stomach. I3C is enzymatically generated from glucobrassicin, an L-tryptophan derived glucosinolate that is enriched in cruciferous vegetables, suggesting a mechanism for immune regulation by dietary components. Kynurenine, an AhR agonist, is a tryptophan metabolite generated by the enzymes indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO). IDO expression is induced by AhR, suggesting positive feedback in this pathway. It has been proposed that activation of AhR with different ligands can lead to different cell fates depending on the surrounding milieu. Microbe-derived ligands can also activate AhR. *Malassezia*, a commensal yeast in human skin, can metabolize tryptophan into several AhR activating compounds, including FICZ and ICZ. *Lactobacillus* converts tryptophan into indole-3-aldehyde (IAld), which can activate AhR and promote IL-22 production by gut ILC3s. Bacterial pigmented virulence factors that are structurally similar to TCDD, such as phenazines produced by *Pseudomonas aeruginosa* and naphthoquinone phthiocol from *Mycobacterium tuberculosis*, have been proposed to bind AhR. Degradation of these virulent pigments is dependent on AhR, as is the inflammatory response by host cells to eradicate these bacterial infections. AhR thus serves dual roles, neutralizing the virulent factors and functioning as a pattern recognition receptor (PRR) that detects these danger-associated molecular patterns (DAMPs) (phenazines/naphthoquinones) and activates host immunity. AhR promotes Th17 cell differentiation from naïve $CD4^+$ T cells (Th17 and Th22).

Kynurenine, a breakdown product in the IDO-dependent tryptophan degradation pathway, has been shown to function as an endogenous AhR ligand and to enhance Treg cell differentiation through the activation of AhR. Another endogenous ligand of AhR (i.e., 2-(1′H-indole-3′-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE)) has also been shown to suppress autoimmunity by inducing Tregs. Treg cells may respond to AhR ligands present in the local tissue milieu, and pathways triggered downstream of this response may be relevant for the maintenance of immune homeostasis via the regulation of both adaptive (Th1/Th17) and innate (dendritic cell (DC) or macrophage (MΦ) responses. The molecular mechanism underlying the development and function of innate-like lymphocytes ILC3s regulated by AhR is incompletely understood. There are at least three mechanisms of action of AhR in ILC3s that have been described. Although, AhR is dispensable for T cell survival, AhR can increase ILC3 survival via the IL-7/IL-7R pathway and anti-apoptotic gene expression,thus promoting ILC3 maintenance. AhR can enhance ILC3 proliferation, leading to its expansion in the gut. AhR has also been shown to directly regulate the transcription of Notch 1 and Notch 2, which are important for ILC3 development. ILCs participate in a dialogue with $CD4^+$ T cells.

It is important that the AhRs are also, or rather primarily, expressed in the neurons of hippocampus and cerebral cortex [Eckers A et al, 2016; Kimura E & Tohyama C, 2017; Ojo ES et al., 2021]. Although AhR expression decreases from the embryonic period into adult life, several physiological functions remain in the adult brain, which include the regulation of synaptic plasticity, neurogenesis, neurotransmitter levels, and blood-brain barrier functions [Wang X et al., 2011; Chen Y et al., 2017; Chen WC et al., 2019; Keshavarzi M et al., 2020].

AhR signaling is considered a promising drug and preventive target, especially in cases of cancer, inflammatory, and autoimmune diseases. Binding of AhR to both xenobiotic and endogenous ligands leads to highly transcriptome-specific cell changes and changes in cellular functions [Esser C & Rannug A, 2015]. It is becoming increasingly clear that the physiological activity of the AhR is nuanced, involving a complex cooperative/competitive "interaction" and changing the AhR from a **toxic mediator to an important sensor of physiological homeostasis** [Murray IA & Perdew GH, 2020; Avilla MN, 2020; Kou Z & Dai W, 2021; Rejano-Gordillo CM et al., 2022].

The discovery of Wang Y et al. [2008] was a new stage in the research of the mechanisms of adaptogenic action of ginseng. It is known that transcriptional activation of the CYP1A1 gene (coding for cytochrome P450 1A1) is mediated by the AhR. The authors have examined the interaction of the ginsenoside Rg1 and Rb1 with the carcinogen activation pathway mediated by the AhR in HepG2 cells. The results showed that in HepG2 cells, CYP1A1 mRNA expression was significantly increased in a concentration- and time- dependent manner by ginsenoside Rg1 and Rb1. Ginsenoside Rg1 and Rb1 activated the DNA-binding capacity of the AhR for the xenobiotic responsive element of CYP1A1. Rg1 and Rb1 were able to activate the ability of the AhR to bind to an oligonucleotide containing the xenobiotic-responsive element (XRE) of the Cyp1a1 promoter. These results indicate that Rg1 and Rb1's effects on CYP1A1 induction are mediated by the AhR. Since CYP1A1 and AhR play important roles in cancerogenesis, development, differentiation, and many other essential physiological functions, these results suggest that the chemopreventive effect of Panax ginseng may be due, in part, to ginsenoside Rg1 and Rb1's ability to compete with aryl hydrocarbons for both the AhR and CYP1A1. Rg1 and Rb1 may thus be natural ligands and substrates of the AhR or have a relationship with AhR pathway. These properties might be helpful for future studies in P. ginseng and chemoprevention in chemical-induced cancer.

Later Hu Q et al. [2013] examined the ability of a series of ginsenosides extracted from ginseng to bind to and activate/inhibit the AhR and AhR signal transduction. The authors

demonstrated the ability of selected ginsenosides to directly bind to and activate the guinea pig cytosolic AHR, and to stimulate/inhibit AHR-dependent luciferase gene expression in a recombinant guinea pig cell line. Comparative studies revealed significant species differences in the ability of ginsenosides to stimulate AhR-dependent gene expression in guinea pig, rat, mouse, and human cell lines. The endogenous gene CYP1A1 could be induced in all cell lines. The authors concluded that the ability of these compounds to stimulate AhR signal transduction demonstrated that these **ginsenosides are a new class of naturally occurring AhR agonists.**

Recently, Zhang M et al. [2024] demonstrated that Low-Medium Polarity Ginsenosides from Wild Ginseng (LWG) improve immunity by reshaping gut microbiota, restoring intestinal mucosa, and boosting the gut microbiota-related metabolism of tryptophan to activate the AhR/MAPK pathway. This research offers new insights into the mechanism by which LWG regulates immune function.

Incidentally, we cannot deny ourselves the pleasure of stating that as early as 1994, the Truskavetsian Scientific School, during a comparative study of the adaptogenic properties of ginseng tincture, the phytocomposition "Balm Kryms'kyi" and Naftussya bioactive water, showed that a four-day treatment of female rats shortened the duration of Nembutal sleep from 159±8 min in control (tap water) to 131±8, 87±8, and 65±5 min, respectively [Panasyuk YM et al., 1994; Alyeksyeyev OI et al., 1996]. This indirectly indicates the activation of microsomal hydroxylation, which is mediated by the cytochrome P450 and AhR complex.

In our opinion, among the given list of organic compounds of Naftussya bioactive water [Datsko OR et al., 2008], there is a high probability that at least one AhR agonist is present. In favor of such an assumption, the data show that AhR, due to the peculiarities of its site, can bind and be activated or inhibited by very different structural compounds [Denison MS et al., 2002; Denison MS et al., 2011; Giani Tagliabue S et al., 2019].

It is interesting that Ozokerite, a component of the standard balneotherapeutic complex of the Truskavets' Spa, has a number of effects similar to those of Naftussya, both when taken orally and when applied to the skin [Popovych AI, 2018; Popovych AI, 2019; Ruzhylo SV et al., 2021], as well as in vitro [Ivassivka SV, 1997]. According to the hypothesis of the Truskavetsian Scientific School of Balneology, polyphenolic compounds of adaptogens of various natures are ligands of AhR [Popovych IL, 2024].

Aryl hydrocarbon receptor is a ubiquitously expressed ligand-activated transcription factor that responds to endogenous and exogenous ligands, such as 6-formylindolo[3,2-b]carbazole (FICZ) and TCDD, respectively. AhR binds to dioxin response element (DRE) sequences in the regulatory regions of target genes and modulates their expression. AhR is expressed and exerts biological functions in several cell types, including immune cells. Due in part to its role in mediating toxic responses to environmental pollutants, AhR activation has not been traditionally viewed as a viable therapeutic approach. Nonetheless, in immune cells, AhR is involved in a variety of processes, such as the xenobiotic response, inflammatory response, antioxidant response, estrogen response, differentiation, and the cell cycle. The expression and activation of AhR can inhibit the proliferation, migration, and survival of cancer cells, and many clinically approved drugs transcriptionally activate AhR. Identification of novel select modulators of AhR-regulated transcription that promote tumor suppression is an active area of investigation. The development of AhR-targeted anticancer agents requires a thorough understanding of the molecular mechanisms driving tumor suppression. Here, we summarize the tumor-suppressive mechanisms regulated by AhR with an emphasis on the endogenous functions of the receptor in opposing cancerogenesis. In multiple different cancer models, the deletion of AhR promotes increased tumorigenesis, but a precise understanding of the

molecular cues and the genetic targets of AhR involved in this process is lacking. The intent of this review was to synthesize the evidence supporting AhR-dependent tumor suppression and distill insights for the development of AhR-targeted cancer therapeutics [Elson DJ & Kolluri SK, 2023].

Recently, AhR has been linked to immune systems through its interaction with the development of natural killer (NK) cells, regulatory T (T_{reg}) cells, and T helper 17 (Th17) cells, as well as the production of immunosuppressive cytokines. However, the role of AhR in carcinogenesis is not as straightforward as we initially thought. Although AhR activation has been shown to promote carcinogenesis in some studies, others suggest that it may act as a tumor suppressor. Congues F et al. [2024] in their review, aimed to explore the role of AhR in the development of cancer that originates from barrier organs. The authors also examined the preclinical efficacy data of AhR agonists and antagonists on carcinogenesis to determine whether AhR modulation can be a viable option for cancer chemoprevention.

Shin JH et al. [2021] showed that in the absence of AhR NK cells have a reduced capacity to migrate and infiltrate tumors in vivo. Their study introduces AhR as a new regulator of NK cell migration through an AhR-ASB2-filamin A axis and provides insight into a potential therapeutic target for NK cell-based immunotherapies. Concerning NK cells, AhR is involved in the regulation of the plasticity between ILC3 and CD56 bright NK cells, the homeostasis of liver-resident NK cells, the anti-tumor response, cytokine production, as well as receptor repertoire expression, including the expression of trafficking receptors. Regarding the migration of immune cells, it was reported that AhR regulates the migration of dendritic cells and regulatory T cells. However, the effect of AhR modulation in other immune cell types, including NK cells, has not been fully assessed. In a previous study, the authors observed that AhR-deficiency was associated with low infiltration of lymphocytes into the tumor microenvironment. Here, they investigated whether AhR regulates the migration of NK cells. The authors found that AhR regulates the expression of ankyrin repeat and SOCS Box containing 2 (ASB2), which encodes the specificity subunit of a multimeric E3 ubiquitin ligase, and that ASB2 regulates the ubiquitination and proteasome degradation of filamin A, which in turn modulates NK cell migration.

Human NK cells are divided into two subsets: $CD56^{bright}$ and $CD56^{dim}$ NK cells, which differ in maturation, function, and distribution. Mechanisms regulating NK cell functions are not completely understood. Moreno-Nieves UY et al. [2018] studied the expression of AhR and its involvement in the regulation of NK cell functions. The authors found that *AhR* mRNA is highly expressed in peripheral $CD56^{bright}$ NK cells and that *AhR* mRNA expression gradually decreases as NK cells display a more mature phenotype. $CD56^{bright}$ NK cells were highly sensitive to AhR ligands. Specifically, AhR ligands modulated their activation and their expression of NK cell receptors, as well as cytokine secretion, which is the major function of these cells. As $CD56^{bright}$ NK cells are highly enriched in tissues and in tumors, the received data point to a possible effect of local AhR ligands in the regulation of the function of $CD56^{bright}$ tissue-resident or intratumoral NK cells.

Nowadays, the AhR has been attributed to multiple endogenous functions to maintain cellular homeostasis. Moreover, crosstalk, often reciprocal, has been found between the AhR and several other TFs, particularly estrogen receptors (ERs) and nuclear factor erythroid 2-related factor-2 (Nrf2). Adequate modulation of these signaling pathways seems to be an attractive strategy for cancer chemoprevention. Several naturally occurring and synthetically modified AhR or ER ligands and Nrf2 modulators have been described. Sulfur-containing derivatives of glucosinolates, such as indole-3-carbinol (I3C), and stilbene derivatives are particularly interesting in this context. I3C and its condensation product, 3,3′-

diindolylmethane (DIM), are classic examples of blocking agents that increase drug-metabolizing enzyme activity through activation of the AhR. Still, they also affect multiple essential signaling pathways in preventing hormone-dependent cancer. Resveratrol is a competitive antagonist of several classic AhR ligands. Its analogs, with ortho-methoxy substituents, exert stronger antiproliferative and proapoptotic activity. In addition, they modulate AhR activity and estrogen metabolism. Their activity seems related to a number of methoxy groups introduced into the stilbene structure [Szaefer H et al., 2024].

Conclusion

Phytotea “ATINE” enhances the immunomodulatory effect of adaptogenic factors of the Truskavets’ spa in patients after radical treatment of oncological pathology. This is probably due to the agonists of aryl hydrocarbon receptors in immune, endocrine, and neural cells present in its composition.

Based on the analysis of the research document on "ATINE" herbal tea used in patients after radical oncological treatment at the Truskavets Spa, the following conclusions supported by mathematical analysis, can be drawn.

1. "ATINE" herbal tea significantly increases NK and T-killer cell levels. Mathematical confirmation: NK cell level increase: standard balneotherapy effect was +0.51±0.09 ($p<0.05$), while with additional "ATINE" it increased to +0.97±0.10 ($p<0.05$); "ATINE" effect alone: +0.46±0.10; T-killer level increase: standard balneotherapy +0.05±0.56 (non-significant), with "ATINE" +1.36±0.46 ($p<0.05$); "ATINE" effect alone on T-killers: +1.31±0.51; Discriminant analysis confirmed these effects with $r^*=0.654$; Wilks' $\Lambda=0.547$; $\chi^2(12)=60$; $p<10^{-6}$.

2. "ATINE" modulates immune response by decreasing T-helper and IgM levels. Mathematical confirmation: T-helper level decrease: standard balneotherapy -0.54±0.28 (non-significant), with "ATINE" -1.16±0.23 ($p<0.05$); "ATINE" effect alone on T-helpers: -0.62±0.26; IgM level decrease: standard balneotherapy -1.47±0.28 ($p<0.05$), with "ATINE" -2.59±0.44 ($p<0.05$); "ATINE" effect alone on IgM: -1.12±0.36; Regression analysis showed strong correlation between T-killer and T-helper level changes: $R=0.909$; $R^2=0.826$; $p<10^{-6}$.

3. "ATINE" increases circulating immune complexes (CIC) without affecting neutrophil bactericidal capacity. Mathematical confirmation: CIC level change: standard balneotherapy -0.12±0.30 (non-significant), with "ATINE" +0.77±0.34 ($p<0.05$); "ATINE" effect alone on CIC: +0.89±0.32; No difference in neutrophil bactericidal capacity (BCCN): standard balneotherapy +1.10±0.58, with "ATINE" +1.06±0.35 ($p<0.05$); t-value for effect comparison: 0.00 (complete absence of difference); Mahalanobis distances between groups confirm significant differences between baseline and both therapies ($p<10^{-6}$), as well as between standard balneotherapy and therapy with "ATINE" ($p=0.075$).

Accordance to ethics standards

Tests in patients are carried out conducted in accordance with positions of Helsinki Declaration 1975 and directive of National Committee on ethics of scientific researches. During realization of tests from all participants the informed consent is got and used all measures for providing of anonymity of participants.

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